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ABSTRACT

This publication is written for those researchers who are interested in the use of radionuclides and radiation in the animal science field. Part I presents topics intended to provide the theoretical base of radionuclides which is important in order to design an experiment for drawing maximum information from it. The topics included in this division are: aspects of ionizing radiation, its detection and measurement, its associated hazards, and some common applications. Laboratory exercises in this division are designed with an aim to provide basic and some advanced skills in this area. Part II, entitled Applied Part, provides a number of detailed practical situations for the use of radionuclides and radiation. There is a small section devoted to exercises on the preparation of radionuclides and procedures with animals. It should provide a systematized course of study for a training program for persons in the field. (Author/PS)

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TECHNICAL REPORTS SERIES No. 60

Laboratory Training Manual on the Use of Radionuclides and Radiation in Animal Research

THIRD EDITION

JOINT FAO/IAEA DIVISION OF
ATOMIC ENERGY IN FOOD AND AGRICULTURE

INTERNATIONAL ATOMIC ENERGY AGENCY, VIENNA, 1972

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LABORATORY TRAINING MANUAL
ON THE USE
OF RADIONUCLIDES AND RADIATION
IN ANIMAL RESEARCH

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A JOINT UNDERTAKING BY THE
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OF THE UNITED NATIONS AND THE
INTERNATIONAL ATOMIC ENERGY AGENCY

INTERNATIONAL ATOMIC ENERGY AGENCY
VIENNA, 1972

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FOREWORD

The use of radionuclides and radiation has proved to be a powerful tool in the agricultural sciences. This manual is designed to give the agricultural researcher the basic terms and principles necessary for understanding ionizing radiation, its detection and measurement, its associated hazards, and some of the more common applications. Basic laboratory experiments to illustrate this purpose are included. Such understanding is necessary if one wishes to gain the most information from the use of radionuclides and to design research experiments for the greatest effectiveness. It is expected that each user of this manual will have had education in basic chemistry, physics, mathematics and statistics as well as his agricultural science speciality.

The further progress of each user will depend on his need and desire to expand his learning by additional reading and training. A list of useful references is included.

The Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA), in co-operation with the Government of the United States of America, Cornell University and Colorado State University, have jointly sponsored several international training courses on the use of radionuclides and radiation in animal research.

Outstanding scientists from various countries have given lectures and devised and conducted the laboratory exercises. Research workers from all over the world have attended these courses in order to apply this experience in their own countries.

The applied part of this present manual, containing a series of detailed laboratory exercises in the use of radionuclides and radiation in animal sciences, represents the efforts of the various instructors who have participated in these training courses.

The first edition of this manual, published in 1966, followed a similar manual (Technical Reports Series No.29) on soil-plant relations research. Since then other manuals (Technical Reports Series No.61, on entomology, and No.114, on food irradiation technology and techniques) have appeared in this series jointly published by FAO and IAEA.

The second edition of the present manual, published in 1968, was enlarged by the addition of several exercises in the basic part.

FAO and IAEA would like to thank all scientists who contributed to the success of the training courses in animal research and in particular Dr. Cyril L. Comar and Dr. Francis A. Kallfelz of Cornell University, who prepared the applied part of the first edition of this manual from lectures given by them and other scientists during the courses. For the additional exercises in the second edition thanks are due to Dr. W. Mulligan of Glasgow University and Dr. F. Lengemann of Cornell University. Dr. James E. Johnson of Colorado State University revised both the basic part and applied part of this third edition.

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SOME BASIC SYMBOLS AND UNITS FREQUENTLY USED IN THIS TEXT

Time (in general)	t
Number of radioactive atoms	N
Radioactive decay constant	λ
Radioactive half-life	$T_{\frac{1}{2}}$
Radioactivity or activity	A
Gamma-ray intensity	I
Number of recorded counts	C
Count rate	r
Unit of count rate	cpm (counts per minute)
Count rate of background	r_b
Count rate of sample plus background	r_{s+b}
Approximately equal to	$\simeq$
Tracer radioactivity	R
Amount of stable tracee	S
Specific activity (R/S)	a
Units of specific activity	μCi/g, cpm/g
Biological half-life	$T_{\frac{1}{2}B}$
Biological rate constant	k
Exchange rate	ρ

BASIC PART

LECTURE MATTER
LABORATORY EXERCISES
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LECTURE MATTER

1. PROPERTIES OF RADIONUCLIDES AND RADIATIONS

1.1. Atomic model: definitions

Radioactive atoms have unstable nuclei. To understand the reasons for this instability, it is necessary to describe the structure of atoms. An atom is composed of a positively charged nucleus which is surrounded by shells of negatively charged (orbital) electrons. The nucleus contains protons and neutrons (collectively termed nucleons) as its major components of mass. Protons are positively charged and have a mass of 1.007277 atomic mass units. Neutrons have no charge and a mass of 1.008665 atomic mass units. Nuclei have diameters of approximately 10^{-12} cm; the diameters of atoms, which include the orbital electrons, are approximately 10^{-8} cm or 1 Ångström unit.

The number of protons in the nucleus (Z) determines the number of electrons and hence the chemical nature of the element. The atoms of a particular element may, however, not all have the same number of neutrons in the nucleus. Atoms that have the same Z but different neutron numbers are called isotopes, because they occupy the same place in the periodic chart. Isotopes, therefore, have identical chemical properties.

As the protons and neutrons represent the major part of the mass of the atom and each has an atomic weight close to unity, the mass number M, which is the sum of the proton and neutron number, is close to the atomic weight. Nuclides (any species of nuclei) are described symbolically by the designation

$$^{M}_{Z}X$$

where X is the chemical symbol for the element.

Nuclei are held together by attractive forces between the nucleons which balance the coulombic repulsion of protons. However, the attractive forces are of shorter range than the repulsive forces and therefore as the atomic number increases, the number of neutrons must increasingly exceed the number of protons. The neutron to proton ratio in the nucleus is the parameter which primarily describes the stability of the nucleus. As the atomic number increases, a point is eventually reached where addition of neutrons is not sufficient to overcome the repulsive forces and above Z = 83 (bismuth) all nuclei are unstable or radioactive. There is also a more or less well-defined optimum neutron to proton ratio for stability of each element. In general, isotopes which have a neutron to proton ratio greater than or less than the stable value for that element are radioactive. All species of radioactive nuclei are called radionuclides.

Radionuclides disintegrate spontaneously, each at a characteristic decay rate. Radioactive nuclei, upon disintegration, may emit alpha (α) or beta (β) particles as well as gamma (γ) rays. These are termed ionizing radiations. Alpha particles are doubly ionized He nuclei ($^{4}_{2}$He), and alpha decay is characteristic of the radioactive heavy elements occurring in nature. Beta particles are electrons of either negative (β^{-}) or positive (β^+) charge, while gamma-rays are electromagnetic radiation of very short wavelength. The energy of electromagnetic radiation is concentrated into packets termed photons.

An example of α-decay is given by the following nuclear reaction

$$^{226}_{88}\text{Ra} \longrightarrow {}^{4}_{2}\text{He} + {}^{222}_{86}\text{Rn} + Q \text{ (energy)}$$

The total energy Q of a nuclear reaction is calculated from the decrease in atomic mass substituted in the equation of Einstein $E = mc^2$, where m is the mass and c is the velocity of light. Since momentum is conserved, the α-particle in this case receives the majority of the kinetic energy.

An excess of neutrons in a nucleus (generally for the lighter elements) can result in β^- decay. An example is

$$^{24}_{11}\text{Na} \longrightarrow {}^{0}_{-1}\text{e} + {}^{24}_{12}\text{Mg} + Q$$

In this case, a neutron in the $^{24}_{11}$Na nucleus is transformed into a proton resulting in an increase in Z number.

A deficiency of neutrons in the nucleus may be counteracted by the emission of a positron. An example is

$$^{65}_{30}\text{Zn} \longrightarrow {}^{0}_{1}\text{e} + {}^{65}_{29}\text{Cu} + Q$$

In this case, a proton in the $^{65}_{30}$Zn nucleus is transformed into a neutron, resulting in a decrease in Z number.

A deficiency of neutrons in the nucleus may alternatively be increased by the capture of an orbital electron. This is termed electron capture (EC) or, commonly, K capture as it describes capture of the K electron. An example is

$$^{85}_{38}\text{Sr} + {}^{0}_{-1}\text{e} \longrightarrow {}^{85}_{37}\text{Rb} + Q$$

This process is accompanied by the emission of characteristic X-rays since the hole in the K shell must be filled by an electron, etc.

After the ejection of a particle, or K capture, the energy of the resulting nucleus may not be at its ground state. The excess energy of this "excited" nucleus is emitted as one or more γ-rays.

A γ-ray may interact with an orbital electron in the decaying atom whereby the electron is ejected from the atom and the photon ceases to exist. This process results in the combined emission of a fast electron and a characteristic X-ray and is known as internal conversion.

In some instances, two alternate modes of decay of the nucleus may occur. An example is

$$^{40}_{19}\text{K} \longrightarrow {}^{0}_{-1}\text{e} + {}^{40}_{20}\text{Ca} \quad (\beta^- \text{ decay, 82\% of disintegrations})$$

$$^{40}_{19}\text{K} + {}^{0}_{-1}\text{e} \longrightarrow {}^{40}_{18}\text{A} \quad (\text{EC, 11\% of disintegrations})$$

In the case of the EC mode a γ-ray also results.

In nature, a number of radionuclides are known and many more can be produced artificially by nuclear fission, by neutron activation or by particle accelerators.

Summarizing, radionuclides will emit particles and/or electromagnetic photons of the following nature:

α-particle	doubly positively charged particle, containing two neutrons and two protons and originating at high speed from the nucleus;
β^--particle	high-speed electron from the nucleus, negatively charged;
β^+-particle	high-speed positron from the nucleus, positively charged;
γ-ray photon	electromagnetic energy packet coming from the nucleus at the speed of light;
X-ray photon	electromagnetic energy packet coming from an electron shell at the speed of light, following K capture or internal conversion;
I.C. electron	(internal conversion electron) electron emitted as a result of the interaction between a γ-ray and a valence electron;
Neutron	particle with no charge and a mass close to that of a proton.

1.2. Radioactive decay

The decay of radioactive atoms is comprised of individual random events. However, if a sample contains a sufficiently large number of atoms of a radionuclide, their average statistical behaviour can be described by precise laws. The radioactive decay law is developed as follows:

Let N = the number of radioactive atoms of a given radionuclide present at any time t. The rate of decay dN/dt is proportional to the number of atoms present or

$$\frac{dN}{dt} = -\lambda N \tag{1}$$

where λ is the proportionality constant, termed the decay constant. The sign is negative because the number N decreases with time.

Rearranging Eq.(1) to solve for λ

$$\lambda = -\frac{dN/dt}{N} \quad \text{or} \quad -\frac{dN/N}{dt} \tag{2}$$

or the decay constant is the fraction of radioactive atoms that decay per unit time.

Equation (1) may be integrated (the steps in this equation are given in Appendix I) to give

$$N = N_0 e^{-\lambda t} \tag{3}$$

where N_0 is the number present at any starting time and N is the number remaining after a period of time t; e is the base of natural logarithms.

It can be seen from Eq.(3) that the decay of radioactive atoms is exponential with time. Also, it follows that the time for N_0 to be reduced to $\frac{1}{2}$ its initial value is a constant independent of N_0.

Let N_0 be reduced to $\frac{1}{2}N_0$ in time t termed the half-life or $T_{\frac{1}{2}}$. Then from Eq. (3)

$$\tfrac{1}{2}N_0 = N_0 e^{-\lambda T_{\frac{1}{2}}} \tag{4}$$

or

$$\frac{1}{2} = e^{-\lambda T_{\frac{1}{2}}} \tag{5}$$

Inverting and taking the natural logarithm of both sides:

$$\lambda T_{\frac{1}{2}} = -\ln \tfrac{1}{2} = 0.693 \tag{6}$$

Thus, the decay constant and the half-life are constants, and are characteristic of a given radionuclide. The $T_{\frac{1}{2}}$ has units of time and the decay constant that of reciprocal time.

Since the disintegration rate, dN/dt, is termed the radioactivity or, simply, activity (A) of the sample, $A = \lambda N$, one can write from Eq. (3):

$$A = A_0 e^{-\lambda t} \tag{7}$$

The half-life of a radionuclide may be determined graphically by plotting the disintegration rate (as determined by a suitable counting instrument) versus time on log-linear co-ordinate paper. Referring to Eq. (7), if one takes the natural logarithm of both sides the result is

$$\ln A = \ln A_0 - \lambda t$$

converting this to common logarithms this becomes

$$\log A = \log A_0 \ \frac{-\lambda t}{2.3}$$

Therefore, plot of A on the log co-ordinate versus time on the linear co-ordinate will be a straight line with a slope of $-\lambda/2.3$. This is graphically illustrated in Fig. 1.

The unit of activity is the curie (Ci) defined as equal to 3.7×10^{10} disintegrations per second or 2.22×10^{12} disintegrations per minute.

Thus, 1 curie (Ci) = 2.22×10^{12} dis/min
1 millicurie (mCi) = 2.22×10^{9} dis/min
1 microcurie (μCi) = 2.22×10^{6} dis/min
1 nanocurie (nCi) = 2.22×10^{3} dis/min
1 picocurie (pCi) = 2.22 dis/min

In practice, radionuclides will often be accompanied by variable quantities of stable isotopes of that element. The stable form is called the carrier. Specific activity is the term used to describe the ratio of radioactive atoms to carrier atoms. The specific activity is defined as the total activity of a particular radionuclide per unit mass of its element

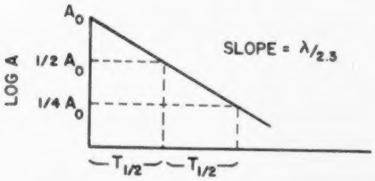

FIG 1. Decay curve of a single radionuclide.

or compound. Common units are μCi/g or μCi/mmole. The specific activity of "carrier free" activity (that is all the atoms of the element present are the same isotope) can be calculated as follows

$$A\ (\mu Ci) = \lambda N\ (\mu Ci)$$

Divide both sides by N, the number of atoms; the result is specific activity in μCi per atom

$$\frac{A}{N}\ \frac{(\mu Ci)}{(atom)} = \lambda\ (time^{-1})$$

If N^0 is Avogadro's number and M is the molecular or atomic weight, then specific activity may be expressed as μCi per gram

$$\text{Specific activity} = \frac{A(\mu Ci) \times N^0(\text{atoms mole}^{-1})}{N(\text{atoms}) \times M(\text{gram mole}^{-1})} = \frac{\lambda N^0}{A} \quad \frac{(\mu Ci)}{(\text{gram})} \quad (8)$$

An example of the production of carrier free and non-carrier free activity will be given in Section 1.4.4.

1.3. The energy of radiations

The energy unit used with regard to radiation is the electron volt (eV). This is equivalent to the kinetic energy acquired by an electron accelerated through a potential difference of one volt. The most commonly used multiple is the unit MeV or million electron volts (10^6eV). One MeV is equal to 1.6×10^{-6} ergs.

The kinetic energies of the particles and photons emitted by radionuclides have characteristic values. The energies of α-particles and X- or γ-rays are constant or discrete. The energies of β-particles, however, ejected by a given radionuclide vary from zero up to a certain maximum energy (E_{max}) that is available to the β-particles. This is because a variable part of E_{max} is taken away by a neutrino in every β-particle decay. Neutrinos cannot be detected by ordinary methods as they have no charge and essentially no mass. As a consequence, the β-particles show a continuous spectrum of energies from zero to E_{max}. The β-energies given in a table or chart of nuclides are E_{max} values; the average β-particle energy is usually about one-third E_{max}. Internal conversion electrons on the other hand are monoenergetic.

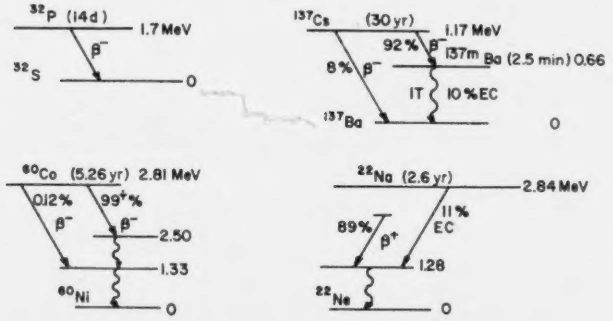

FIG. 2. Decay schemes showing characteristic radiations and energies of four different radionuclides.
IT = Isometric transition. Different types of the same nucleus are called isomers.
EC = Electron capture, K capture.

The characteristic radiations and energies for a given radionuclide are often shown in the form of decay schemes. Examples of the decay schemes of four commonly used radionuclides are shown in Fig. 2.

1.4. Interaction of radiation with matter

1.4.1. Interaction of alpha particles with matter

The α-particles ejected from any particular radionuclide are monoenergetic. Their initial kinetic energies are in the order of several MeV and, since ionization potentials and bond energies are in the range 1-12 eV, the α-particles are capable of causing ionization as well as electronic excitation of the atoms or molecules along their path. Ionization is complete removal of the valence electron and excitation is raising electrons to higher energy levels in their orbits. Since the valence electron participates in any chemical bond of the atom, ionization destroys the integrity of that bond. Alpha particles are doubly charged and of comparatively heavy mass and, therefore, form a dense track of ion pairs (i.e. ejected electrons and positively charged ions) along their path. Therefore, they lose energy relatively rapidly in matter by these processes. As the α-particle dissipates its energy along its path the velocity of the particle decreases and at zero kinetic energy the particle acquires two electrons from its surroundings and becomes a helium atom. The range, i.e. the distance that an α-particle can penetrate into any matter (absorber) depends on the initial energy of the particle and the density of the absorber. The range of an α-particle is relatively small and amounts to several centimetres in air and several microns (10^{-3} mm) in tissue for energies in the order of 1 - 10 MeV.

1.4.2. Interaction of beta particles with matter

Beta particles lose energy in matter by ionization and excitation just as do α-particles. The mass of the β-particle, however, is only 1/7300

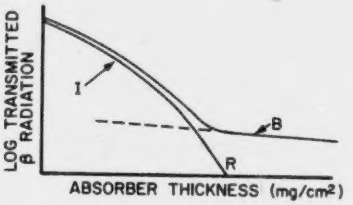

FIG. 3. Curve demonstrating the transmitted β-radiation as a function of absorber thickness.
I = Intensity of transmitted β-radiation.
B = Bremsstrahlung component (and γ-ray component).
R = Approximate range of β-particles in absorber material.

of the mass of the α-particle and β-particles have only unit charge. They will, therefore, scatter more, penetrate further into matter and produce a less dense track of ion pairs than will α-particles. The range of β-particles in matter is also a function of the initial energy of the particle and the density of the absorber, but this range is not so well defined because of the zig-zag path (due to scattering) of the electron. The range of β-particles of 1 MeV initial energy is approximately 300 cm in air and 0.4 cm in tissue.

Because of the fact that β-particles have a continuous spectrum of energies up to E_{max}, their absorption in matter is approximately exponential with absorber thickness. Therefore, when the β-radiation transmitted by an absorber is plotted as a function of absorber thickness on semi-log paper, a fairly straight line is obtained over a portion of the curve (Fig. 3).

The curve becomes reasonably flat at R, approximately the range for β-particles with E_{max}. Although nearly all the β-particles are stopped by this thickness of absorber, one still finds transmission of radiation, because the β-particles interact with the atoms of the absorber giving rise to non-characteristic X-rays, or bremsstrahlung. In addition, any γ-rays will contribute to this component. By subtracting this component from the composite curve, the pure β-transmission curve I is obtained.

Positive β-particles, termed positrons, lose their kinetic energy in matter in exactly the same manner as negative β-particles. However, when the kinetic energy of the positron has been reduced to zero by ionization and excitation, the positron is annihilated together with an electron giving rise to two annihilation photons of 0.51 MeV each. (0.51 MeV is the equivalent energy of the rest mass of an electron.)

Absorption and scattering of β-particles are important in their measurement in samples. Absorption and scattering will occur in the sample cover, the detector window, the walls of the shield, the intervening air and in the sample itself. These effects will all influence the counting rate, self-absorption being the most important. This will be illustrated by Laboratory Experiment 1.4.

1.4.3. Interaction of gamma rays and X-rays with matter

Electromagnetic radiation is considerably more penetrating than particulate radiation of the same energy. This is because the electromagnetic radiation must first undergo an absorbing event to produce a "secondary" ionizing particle before its energy may be dissipated. Gamma rays will be absorbed in matter as a function of their energy, the Z, and the density of the absorbing material. (γ-rays and X-rays differ only as to their origin and interact identically in matter). Three types of absorbing event may occur:

(1) photoelectric absorption

(2) Compton absorption

(3) pair-production absorption

(1) Photoelectric absorption is predominant for low-energy γ-rays and for absorbers of a high Z material. The γ-ray interacts with a K or L electron of the absorber atom and ejects it from the atom with a kinetic energy equal to the initial γ-ray energy minus the binding energy of that K or L electron. Thus, an electron is produced with kinetic energy to produce ionizations and excitations along its path exactly in the manner of a β-particle.

In Fig. 4, the coefficient for photoelectric absorption is given for water as a function of γ-ray energy. The absorption coefficient is a measure of the probability of absorption.

(2) Compton absorption is the interaction of the γ-ray with an outer electron of the absorber atom. Part of the initial kinetic energy is transferred to this electron and the γ-ray photon is scattered off in a new direction at a lesser energy. As can be seen from Fig. 4, this effect is at a maximum in water for γ-rays of about 0.5 MeV. The effect rises only slightly with increasing Z. The electron ejected will produce ionization and excitation again exactly in the manner of the β-particle.

(3) When the γ-ray has an initial energy of 1.02 MeV or greater it may undergo pair-production absorption. In this process, the γ-ray interacts with the positive field of the nucleus of the absorber atom and is completely annihilated producing a positron-electron pair. Since it requires 1.02 MeV for this pair formation, this is the threshold for this event. Any γ-ray energy above this required 1.02 MeV is imparted as kinetic energy to the positron-electron pair. Both the positron and the β-particle cause ionization and excitation along their respective paths. The two 0.51-MeV photons produced upon annihilation of the positron must be absorbed by Compton or photoelectric events or a combination of the two. In Fig. 4, the absorption coefficient for pair-production is labelled as K.

Considering the above processes, a beam of monoenergetic γ-rays is absorbed exponentially as a function of thickness of the absorbing material. For a beam of intensity I the change in intensity per unit absorber thickness, dI/dx, is proportional to the intensity of the beam.

$$\frac{dI}{dx} = -\mu I \qquad (9)$$

This is identical to the well-known Lambert-Beer law for attenuation of monochromatic light. The proportionality constant μ is termed the total linear attenuation coefficient. Exactly analogous to radioactive decay, μ is the fraction of the original intensity removed from the beam per unit thickness. Equation (9) is identical to the radioactive decay law and may be integrated to give

$$I = I_0 e^{-\mu x} \tag{10}$$

where $\mu = \mu_s \ \mu_a$
and μ_a = total absorption coefficient
μ_s = total scattering coefficient
$\mu_a = T + \sigma_a + K$
T = photoelectric absorption coefficient
μ_a = Compton absorption coefficient
K = pair-production absorption coefficient.

Fig. 4 illustrates these absorption probabilities for water as a function of E_γ.

Again analogous to radioactive decay the thickness at which I_0 is reduced to one-half its initial intensity is termed the "half-thickness" or "half-value layer" ($X_{\frac{1}{2}}$).

$$X_{\frac{1}{2}} = \frac{0.693}{\mu} \tag{11}$$

An understanding of gamma-ray interactions is necessary in considerations of shielding, dose calculations and measurement of γ-rays.

1.4.4. Neutron production and interaction with matter

Neutrons have no charge and, therefore, cannot ionize directly. However, they can produce ionization indirectly and are generally considered in discussions of ionizing particles

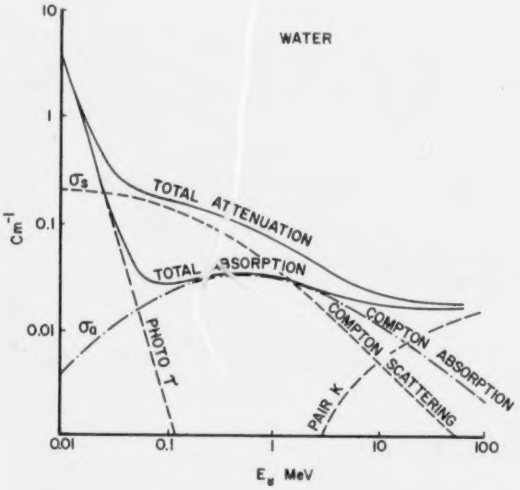

FIG. 4. Linear attenuation coefficients for γ-rays in water.

Neutrons are produced by the fission reaction and the most common sources of neutrons are nuclear reactors which control the fission chain reactions. Neutrons can also be produced by small laboratory sources, and these sources will be discussed in greater detail in Section 7.1.

Neutrons lose energy and interact with matter by the following processes:

(1) Elastic collisions. Neutrons of high initial energy "fast neutrons" interact with other nuclei in billiard-ball-like collisions, losing a fraction of their kinetic energy per collision. By this moderation process they eventually reach an energy which is the same as molecules in thermal equilibrium with their particular environment. Hence they are then termed "thermal" neutrons. The light elements, especially H, are the most efficient for this moderating process.

A fast neutron undergoing an elastic collision with another atom or molecule will produce generally a fast-recoil ionized atom. This recoil ion will then produce ionization and excitation along its path.

(2) Absorption reactions. Examples are given below of the four principal types of absorption reactions. These occur predominantly with slow or thermal neutrons:

(a) $$^{59}_{27}\mathrm{Co} + {}^{1}_{0}\mathrm{n} \longrightarrow {}^{60}_{27}\mathrm{Co} + \gamma$$

(n, γ) reaction. This type of reaction is used to produce many of the artificial radionuclides. In the example, the ^{60}Co produced cannot be chemically separated from the stable ^{59}Co in the sample, and this is an example of production of non-carrier free activity.

(b) $$^{14}_{7}\mathrm{N} + {}^{1}_{0}\mathrm{n} \longrightarrow {}^{14}_{6}\mathrm{C} + {}^{1}_{1}\mathrm{H}$$

(n, ρ) reaction. This is the reaction by which cosmic-ray neutrons produce ^{14}C activity in the biosphere. This reaction is also used to produce ^{14}C commercially and, since the ^{14}C can be chemically separated from the nitrogen compound in the sample, it is an example of the production of carrier free activity.

(c) $$^{235}_{92}\mathrm{U} + {}^{1}_{0}\mathrm{n} \longrightarrow {}^{90}_{36}\mathrm{Kr} + {}^{144}_{56}\mathrm{Ba} + 2\ {}^{1}_{0}\mathrm{n}$$

(n, fission) reaction. This illustrates fission of ^{235}U into two fission fragments and two additional neutrons.

(d) $$^{10}_{5}\mathrm{B} + {}^{1}_{0}\mathrm{n} \longrightarrow {}^{7}_{3}\mathrm{Li} + \alpha + \gamma$$

(n, α) reaction. This is a reaction commonly used to detect neutrons.

2. RADIATION DETECTION

The reactions that the various types of radiation produce in matter can be used to detect and to measure the radiation. Of these reactions, the most commonly used are ionization in gases, orbital electron excitation in solids or liquids and specific chemical reactions in sensitive emulsions. The most common types of detection methods employing these mechanisms are described below.

2.1. Autoradiography

This method is a photochemical process and the one used by Becquerel in 1896 in the discovery of radioactivity. Ionizing radiations interact with the silver halide in photographic emulsions. When radioactive material is placed on a photographic plate or film, a blackening will be produced on development of the emulsion. The blackened areas constitute a "self-portrait" of the activity in the material. The intensity of the blackening (as determined with a densitometer) at a given place will be a function of the exposure time and the amount of activity in the sample at that place. It also is a function of the linear-energy transfer of the particular radiation. The linear-energy transfer (LET) is the energy lost by the particle per unit path length. Gamma-rays must first be absorbed to produce an ionizing particle and will produce very little blackening. On the other hand, α-particles and low-energy β-particles which have a high LET (^{3}H, ^{14}C, ^{35}S, ^{45}Ca) are very effective. High-energy β-particles produce diffuse radiograms due to the relatively long path lengths of these particles in the emulsion. The properties of the emulsion should be a compromise between fine grain to increase the resolution, and high sensitivity to reduce the exposure time. Usually, exposure times are long. For example, a thin histological section containing about 100 dis/min per cm^2 will require several weeks exposure to show sufficient blackening for accurate measurement.

The method of autoradiography is particularly suitable when the distribution of a radioactive compound in biological material is to be studied. Autoradiography is essentially a method to detect radiation and not generally suitable for measurement or quantitative purposes.

2.2. Ionization detectors

A number of detectors are based on the principle that the ions produced by radiation in a gas will migrate towards the appropriate electrodes in an electric field. If the potential difference between the two collecting electrodes is sufficiently large then the ion pairs formed by the particle will not recombine but be collected at an electrode to produce an ion current. If the ion current is amplified and measured, it is proportional to the activity of the sample being measured.

A simplified ionization chamber circuit is shown in Fig. 5.

The chamber is filled with air and the collecting electrode is connected through a resistor to the positive side of the power supply. The collecting electrode or anode is insulated from the walls of the chamber which serve as the cathode. If now ionizing particles traverse the chamber, ion pairs will be formed along their paths. The electrons produced will collect on the central electrode and the ion current is measured by special electrometer circuits.

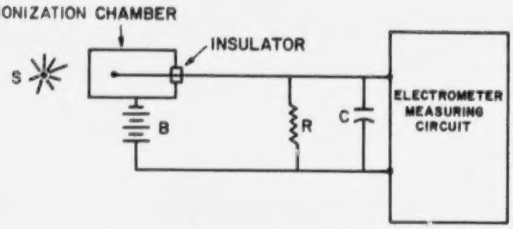

FIG. 5. Schematic diagram of ionization-chamber circuit.
B = Potential source
R = Resistor
C = Capacitor
S = Radioactive source

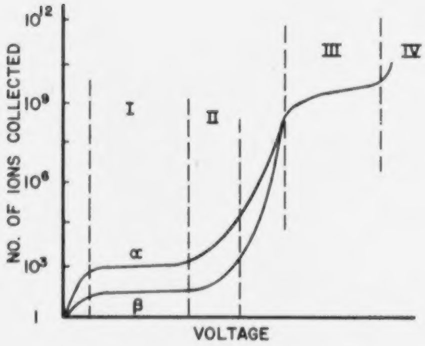

FIG. 6. Plot of ionization versus counter-tube voltage. I is the ionization-chamber region; II is the proportional region; III is the Geiger-Müller region; IV is the region of continuous discharge.

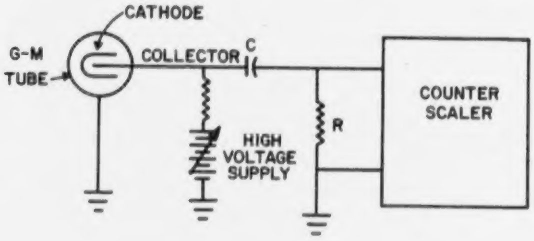

FIG. 7. Counter input circuit with a G-M tube detector.

As will be seen in Section 3.2.1, if the walls of the ionization chamber are constructed of "air-equivalent" material, such an ionization chamber may be used to measure exposure dose of γ-rays or X-rays in air.

If, however, now the electrode potential is increased, then the ions produced will move toward their respective electrodes with greater velocity and at some voltage will gain sufficient kinetic energy to produce further ionization themselves. This process is known as gas amplification and the flood of ions produced is termed the Townsend Avalanche. As a result of gas amplification, the pulse size produced increases rapidly with applied voltage. Figure 6 shows the relationship of ion production to applied counter voltage.

The graph is drawn for an α-particle and a β-particle traversing in the sensitive volume of the tube.

In region I, the applied voltage is sufficiently great that all ions formed are collected. This is called the ionization chamber region.

As the applied voltage is increased, amplification results and in region II the pulse size produced is proportional to the number of ion pairs produced in the initial event. This is termed the proportional region. In region III, the voltage is sufficiently great that the size of the charge collected on the anode is no longer dependent on the number of primary ions produced. In this region then at a given voltage all pulses are the same size and this is termed the Geiger-Müller (G-M) region. Detector tubes operated as Geiger-Müller tubes are very sensitive and very little additional amplification of the pulse is necessary to drive a counting circuit. In addition, they are relatively inexpensive. A simplified G-M counter circuit is shown in Fig. 7.

The discharge in a G-M tube would continue indefinitely or until all the gas molecules were ionized. Generally certain gas molecules are added to quench the discharge. For example, noble gases such as argon are added and these dissociate when they collide with positive ions rather than become ionized themselves. In this fashion, the discharge is stopped and the noble gas atoms then recombine.

The fact that some time is required to collect the flood of electrons from each discharge and for the quenching process implies that during this period the detector tube will be insensitive to other ionizing particles in its sensitive volume. This period is approximately 100-300 μsec for G-M tubes and is termed the resolving time. Therefore, particularly at high count rates a correction for this resolving time must be made.

Let r be the observed count rate of a G-M counter and τ the resolving time of the counter in minutes. During one minute, the counter will have been ineffective for $r\tau$ min. Therefore, r counts have been registered in only $(1 - r\tau)$ min. The true count rate (r_{true}) therefore is

$$r_{true} = \frac{r}{1-r\tau} \frac{(\text{counts})}{(\text{min})} \text{ or cpm} \qquad (12)$$

A method for calculating the resolving time of a counter is given in Experiment 1. Correction is normally not necessary unless the count rate exceeds about 2000 cpm.

The necessary associated equipment for a G-M counting system include the G-M tube, a power supply, an amplifier-discriminator, a scaler and a timer.

2.3. Detection by excitation

2.3.1. Solid scintillator counting

Solid scintillators are particularly suited for the detection of γ-rays and X-rays because of the high density and high Z of solid crystals. The alkali halides (particularly NaI activated with Tl) have been the most useful. A typical scintillation crystal counter system is diagrammed in Fig. 8.

When a γ-ray photon is absorbed in the scintillation crystal at least one fast electron is liberated (depending upon the absorbing event either a photoelectron, Compton electron or pair-production electrons). These electrons produce excitation and ionization along their paths in the crystal. When the excited atoms return to the ground state they give rise to light photons in the violet or near-ultraviolet range. The number of light photons produced will be proportional to the γ-ray energy lost in the crystal.

The photocathode of the photomultiplier tube is optically coupled to one face of the scintillation crystal and the photons produced are reflected inside the crystal until they reach the photocathode. Here, by the photoelectric effect, they release photoelectrons. The number of photoelectrons again is proportional to the γ-ray energy lost in the crystal.

In the photomultiplier tube the photocathode is connected to a series of dynodes or electrodes each at a successively higher positive potential than the preceding stage. Thus, photoelectrons released from the photocathode surface will be attracted to the first dynode stage and will gain sufficient kinetic energy to release two or more secondary electrons from the first dynode. This multiplication process occurs at each stage and at the end of 10 or more stages in a typical photomultiplier tube a large pulse of electrons will arrive at the anode. The size of this pulse still will be proportional to the original γ-ray energy lost in the crystal. The pulse is then amplified linearly and directed to a scaler or pulse-height analyser.

In the pulse-height analyser, the pulses are sorted by virtue of their voltage size (pulse-height) and stored in the respective portion of an electronic memory. After counts have been collected for a period of time, the readout of the memory will be a γ-ray spectrum of the activity available to the scintillation detector.

The energy lost in the scintillation crystal from an incident γ-ray photon will range from zero to its maximum energy depending upon the absorption event. The γ-ray can be absorbed by the photoelectric effect or by a Compton interaction followed by photoelectric absorption of the scattered photon or by any combination of processes that lead to total absorption of the γ-ray energy within the crystal. If this occurs then the output pulse will be stored in a location proportional to the γ-ray energy. A typical γ-ray spectrum is shown in Fig. 9 and the resulting peak is labelled as the total absorption peak. If, however, the primary interaction in the crystal is Compton absorption and the scattered photon escapes from the detector, then the energy deposited in the crystal will be less than the energy of the γ-ray. The range of possible Compton interactions result in a distribution of pulse heights. This distribution is labelled as the Compton region in Fig. 9. It is apparent then that the

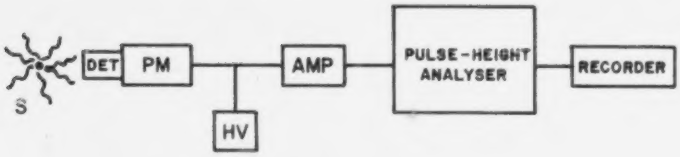

FIG. 8. Block diagram of NaI(Tl) scintillation counter system.
S = Gamma-ray source
DET = NaI(Tl) scintillation detector
PM = Photomultiplier tube
HV = High-voltage power supply
AMP = Linear amplifier.

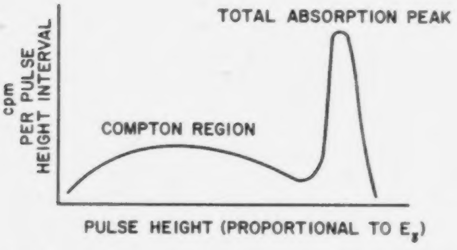

FIG. 9. A γ-ray spectrum of radionuclide emitting a single γ-ray.

location of the total absorption peak is characteristic of the particular γ-ray emitter and is useful in identification of the γ-ray emitters in any sample. The area under the total absorption peak is proportional to the activity of that radionuclide in the sample. The peak is broadened into a distribution due to statistical variation in the several conversion steps from γ-ray absorption to final pulse-height.

Pulse-height analysis is only required when it is necessary to measure the activity of one γ-ray emitter in the presence of others, e. g. in neutron-activation analysis or multiple-tracer experiments. Generally a less expensive single-channel, pulse-height analyser is sufficient.

The other distinct advantage of solid scintillation counting is the very short resolving time of such systems. This enables high count rates to be determined (up to at least 100 000 cpm) without the necessity of resolving time correction.

Additional details of γ-ray spectral analysis will be presented in Experiment 2. 1.

The art of particle and γ-ray spectroscopy has recently been significantly advanced by the development of semiconductor radiation detectors. In particular, lithium-activated germanium detectors have been very useful in γ-ray spectroscopy. The energy resolution of such systems is improved by at least a factor of 10 over NaI(Tl) for ^{137}Cs γ-rays. Semiconductor detectors have the disadvantage that they cannot be made as large as NaI(Tl) and therefore the counting yield is decreased. Also, they must be continually maintained at very low temperature. In neutron-activation analysis, for example, where high γ-ray resolution is necessary, use of such detectors is warranted.

2.3.2. Liquid scintillation counting

Liquid scintillation counting techniques have promoted application of radionuclides in the agricultural and biological sciences because they have allowed much wider use of low-energy β-particle emitters such as ^{3}H and ^{14}C.

In this technique, the sample to be counted is placed in solution with an organic scintillating material in an organic solvent. Since each radioactive atom or molecule is surrounded by molecules of the scintillator, self-absorption is greatly reduced and the counting yield greatly increased. If the sample is insoluble in the organic solvent often it may be put in uniform suspension.

There are now many solute-solvent liquid scintillator systems in use; however, a very common one is PPO (2-5 diphenyloxazole) with toluene or dioxane as the solvent.

The ionizing particle from the radioactive material causes excitation and ionization of the solvent molecules. These transfer their excitation energy to the PPO molecules which in turn fluoresce, i.e. give rise to a light photon in the return to ground state. The number of light photons emitted from the counting vial per ionizing particle is proportional to the energy lost by that particle in the solution. The counting vial is optically coupled to a photomultiplier tube system to collect the output light. A block diagram of a liquid scintillation system is shown in Fig. 10.

Two photomultiplier tubes commonly are used to collect the light output from the scintillation vial. This increases the counting yield and from a single ionizing event the light photons will be registered at

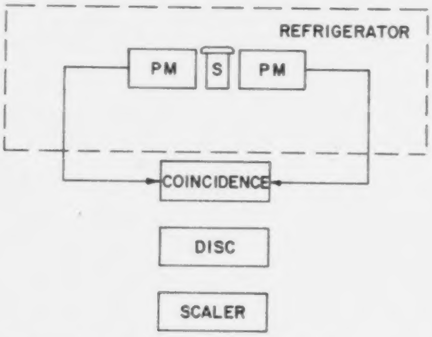

FIG. 10. Block diagram of typical liquid scintillation counter.
S = Counting vial containing liquid scintillation solution and sample
PM = Photomultiplier tubes
Refrigerator = Refrigerating unit at -5°C
Coincidence = Prompt coincidence and sum circuit
Disc = Upper- and lower-level discriminator.

both photocathodes simultaneously. The coincidence circuit is designed to produce one output pulse if it receives an input pulse from each photomultiplier simultaneously, or "in-coincidence". Background or noise pulses from either tube will then not be in-coincidence, and will be rejected. This system increases the ratio of true counting rate to background and increases the sensitivity. Since the output pulse height is proportional to the energy lost in the liquid scintillator, limited pulse-height analysis is possible. It is limited because the pulse-height resolution is very poor. However, it is generally possible to count both ^{3}H and ^{14}C simultaneously.

The detector part of the system is often refrigerated to reduce thermally produced noise in the photomultiplier tubes.

For certain high-energy β-emitters ($E_{max} > 1$ MeV) it is often possible to employ Čerenkov counting techniques using the liquid scintillation counter. In such cases, the radioactive sample need only be dissolved in water. The ionizing particle travels through the water at velocities greater than the velocity of light in that medium and Čerenkov light is produced. The amount of light is proportional to the activity of the sample. Čerenkov counting techniques have proved useful with ^{42}K, ^{24}Na and other high-energy β-emitters.

One of the main sources of error in liquid scintillation counting is "quenching" of the light caused by compounds in the solution to be measured. This can be due to light absorption by coloured compounds or by certain chemicals. Since quenching commonly occurs and its degree can be variable, it must always be considered. The three most important correction methods are listed below:

(1) Removal of coloured material. The solution may be filtered through activated charcoal or treated by an ion-exchange technique to remove the quenching agent.

(2) Channels ratio method. In general, quenching is greatest for the highest-energy photons. Therefore, when quenching occurs, the output pulse spectrum is shifted to lower energy or longer wavelengths. If by discriminator settings the ratio of two parts of the spectrum can be obtained, then it is possible to observe the shift in this ratio as a function of the counting yield in a set of standards with a known amount of activity and known variable amounts of quench. A standard curve can then be used to correct each count rate for the decrease due to quench.

(3) External standard technique. In some instruments a standard source may be moved into position beneath the vial counting position. Thus, the relative decrease in standard count for each sample counting vial will be proportional to the amount of quenching material and will provide the quench correction to be used for that vial count.

2.4. Counting yield

Practically every tracer experiment involves a number of samples containing radioactivity, and the assay of the activity of these samples is an integral part of the complete experiment. When a radioactive atom decays, often more than one particle or photon is emitted. For example, a ^{60}Co nucleus emits either one β-particle and two γ-rays or occasionally one of each (see Section 1.3). However, metastable states excepted, a

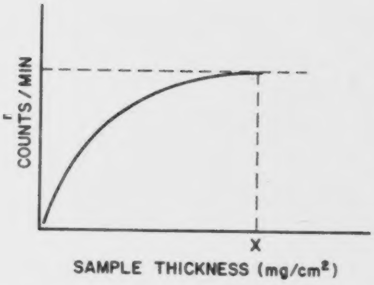

FIG. 11. Count rate as a function of thickness for samples of constant specific activity.

disintegration including the emission of particle(s) and/or photon(s) requires only 10^{-10} sec or less, whereas the dead times of even the fast counters are in the order of 10^{-7} sec. Thus, no practical counter will have a counting yield, Y counts per disintegration, of greater than 1.

$$\text{Counting yield } Y = \frac{\text{count rate}}{\text{disintegration rate}} = \frac{r\ (\text{counts/min})}{A\ (\text{disintegrations/min})} \qquad (13)$$

In most counters the counting yield is considerably less than 1, that is only a fraction of the total disintegrations of the sample are detected and registered by the counting system. Y is decreased from 1 due to:

(1) Geometry considerations: the solid angle of the source detector arrangement divided by 4π. For a small source close to the detector window the solid angle is about 2π and the geometry factor about 0.5.

(2) Air and window absorption: particles, particularly α and low-energy β, or photons may be absorbed in the air, the window or walls of the detector and never reach the sensitive volume of the detector.

(3) Self-absorption in the sample: α- and β-particles, and to a much lesser extent γ-rays, can be absorbed by the material in which the radionuclide is contained and a significant fraction of the activity will not be counted. This is a very important consideration for low-energy β-particles. In consequence, the count rate from a given sample will not increase in proportion to its thickness. For a sample of a given area, as sample thickness of constant specific activity material increases, the count rate will reach a maximum as in Fig. 11. At thickness X the sample is considered to be at "infinite thickness". Thickness is expressed as the product of density and linear thickness and has units of mg/cm^2. A common method for assay of low-energy β-emitters using G-M counting is to count all samples at "infinite thickness". The count rate is then proportional to the specific activity. The value for infinite thickness of β-emitters is approximately equal to the range of β-particles in units of mg/cm^2. Reference [4] contains a curve of β-particle range as a function of energy.

(4) Scattering: particles or photons may be scattered towards or away from the sensitive volume of the chamber. This scattering is due to the backing material of the sample holder, the walls of the shield and the air between the source and the window.

In most applications, the counting yield need not be determined by investigating each of the above effects individually. Instead a standard, i. e. a source of known activity or disintegration rate, prepared in identical fashion with the samples, is counted under the same geometry to determine the counting yield. Such standards may be purchased from radionuclide suppliers or may be prepared from the radioactive material to be used in the experiment. In the latter case, the count rate of all experimental samples can be compared as a fraction of the experimental amount administered.

2. 5. Counting statistics

If a single radioactive sample is counted several times under identical conditions and the count rate is corrected for radioactive decay or else the decay correction is negligible, the individual count rates will be observed to deviate about the mean value. These deviations are due to the random nature of radioactive decay (Section 1. 2). The understanding of these statistical effects is necessary in the consideration of experimental design and in the interpretation of experimental results.

Counting statistics follow closely the Poisson probability distribution. By a special property of the Poisson distribution, the standard deviation (σ) of a registered number of counts C, is equal to the square root of that number. For C registered counts

$$\sigma = \sqrt{C} \qquad (14)$$

Table I gives the calculated deviation for given accumulated counts. As can be seen from the table, the standard deviation increases as the square root of the number of counts but decreases as a percentage of the counts.

Referring to Eq. (14), if both sides are divided by the period of counting t, the result is the standard deviation of the count rate r.

$$\frac{\sigma}{t} = \frac{\sqrt{C}}{t} = \sigma_r \qquad (15)$$

However, since C = rt

$$\sigma_r = \frac{\sqrt{rt}}{t} = \sqrt{\frac{r}{t}} \qquad (16)$$

When C becomes large (>20), the Poisson distribution is closely approximated by the normal distribution. From the normal distribution, one standard deviation on either side of the mean value accounts for 68. 3% of the total area under the probability function.

A useful rule of counting is to try to accumulate at least 10 000 counts, in order for the % standard deviation to be 1% or less. If 10 000 counts exactly are accumulated, then from this single assay it can be stated that there is a 68. 3% probability that the true total count was 10 000 ± 100,

TABLE I. STANDARD DEVIATION OF ACCUMULATED COUNTS

Accumulated counts C	Standard deviation $\sigma = \sqrt{C}$	Standard deviation as % C
100	10	10
1 000	31.6	3.2
10 000	100	1.0
100 000	316	0.3
1 000 000	1 000	0.1

or in the range 9900 to 10 000. Two standard deviations (2σ) account for approximately 95% of the area under a normal distribution and in this case it can be stated that there is a 95% probability that the true total count was 10 000 ± 200.

The number of counts C collected in any counting interval are due to true counts of the sample plus those due to background. There is a significant radiation background in almost any location. This background is due to cosmic rays and to cosmic-ray induced activity, such as ^{14}C, and to naturally occurring radioactive materials in the earth's crust, e.g. ^{226}Ra, ^{232}Th and ^{40}K. The latter all have associated gamma rays. The cosmic-ray contribution varies with altitude and the composition of the earth's crust varies with location. All radiation detector-counter systems have an associated background counting rate due to the above sources and also electronic noise. The background count rate is commonly reduced by shielding or by special electronic circuitry.

Obviously, every sample count is made in the presence of a background counting rate for that particular system. The background will be a function of the type of detector, the shielding, location, discriminator settings, etc.

The variations of background are independent of the variations of the sample activity and the appropriate error terms add as the sum of the squares. Therefore, the variance due to the sample activity alone is

$$\sigma_s^2 = \sigma_{s+b}^2 + \sigma_b^2 \qquad (17)$$

where σ_s^2 = variance due to sample alone
σ_{s+b}^2 = variance due to count of sample plus background
σ_b^2 = variance due to count of background

For a total of C accumulated counts due to sample and background counts the standard deviation of the sample count is given from Eqs 14 and 17 by

$$\sigma_s = \sqrt{C_{s+b} + C_b} \qquad (18)$$

where C_{s+b} = total counts due to sample plus background
C_b = total counts due to background counted alone

If t_s = counting time for sample in the presence of background and

t_b = counting time for background,

then the standard deviation of the sample counting rate is given by

$$\sigma_{r_s} = \sqrt{\frac{r_{s+b}}{t_s} + \frac{r_b}{t_b}} \qquad (19)$$

In tracer experiments, the net counting rate of samples very commonly approaches, or is even less than, background counting rates. In order to choose the best division of time for counting sample and for counting background to obtain the minimum error the following may be used:

$$\frac{t_b}{t_s} = \sqrt{\frac{r_b}{r_{s+b}}} \qquad (20)^1$$

2.6. Neutron detection and measurement

Nearly all the interactions that neutrons undergo in matter are used in their detection and measurement. Fast neutrons by collision with other nuclei produce charged recoil nuclei which can cause ionization. Slow and thermal neutrons undergo absorption reactions to produce charged particles or induced radioactivity.

The most common method of slow and thermal neutron detection is use of a counting tube filled with boron trifluoride gas or lined with boron inside the tube. Ionization is produced in the tube gas by the nuclear reaction:

$$^{10}_{5}\mathrm{B} + {}^{1}_{0}\mathrm{n} \longrightarrow {}^{4}_{2}\mathrm{He}\ {}^{7}_{3}\mathrm{Li} + \gamma$$

Both the α-particle and the $^{7}_{3}$Li recoil nucleus produce ionization. The counter tube is generally operated in the proportional region to give discrimination from pulses that may be produced from interfering γ-rays. The tube can be used to detect fast neutrons by surrounding it with paraffin wax to moderate the fast neutron to slow or thermal energies.

A BF_3 detector is used for the detection of slow neutrons in the determination of moisture content in materials. A source containing a mixture ^{239}Pu and Be (or ^{241}Am and Be) provides fast neutrons which are moderated or slowed down by the hydrogen atoms in water. Thus, the count rate of the tube due to slow neutrons is proportional to the water content.

Activity induced in materials also is commonly used for neutron detection. An end-window G-M tube with a piece of silver foil across the window may be used to monitor neutron radiation. The neutrons activate silver atoms to radioactive isotopes which emit β-particles that are detected by the G-M tube. After several minutes, the count rate of the G-M tube is proportional to the neutron flux.

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[1] Derivation of Eq. (20) is given in Appendix II.

3. RADIATION PROTECTION

It is imperative that a knowledge of the safe use of radionuclides and radiation be gained before they can be applied as tools in agricultural research. Ionizing radiation is hazardous to all biological systems, but with proper consideration for health protection measures, the hazard to personnel or experimental systems can be insignificant.

3.1. Units and basic considerations

The Röntgen (R) is the unit describing exposure of X- or γ-rays in air. It is defined as the quantity of X- or γ-radiation which produces, in air, ion pairs carrying 1 electrostatic unit of charge of either sign per cm^3 of air at STP. On the average, it requires 33.7 eV to produce one ion pair in air. Therefore

$$1\ \text{R} = \frac{(1\ \text{esu/cm}^3)\ (33.7\ \text{eV/ion pair})\ (1.6 \times 10^{-12}\ \text{ergs/eV})}{(1.29 \times 10^{-3}\ \text{g/cm}^3)\ (4.8 \times 10^{-10}\ \text{esu/ion pair})}$$

$$= 87.7\ \frac{\text{ergs}}{\text{gram of air}} \qquad (21)$$

Therefore, 87.7 ergs are absorbed per gram of air due to a total exposure of 1 R.

As discussed in Section 1.4.3, the energy of γ-rays absorbed per gram of various materials is a function of properties such as Z and density. Therefore, the energy absorbed from exposure to 1 R will be slightly different for soft tissue and for bone as compared to air and would be a function of photon energy.

Any biological effect from a given type of radiation is proportional to the energy absorbed and the basic unit of absorbed dose is termed the rad. One rad is equal to an absorbed dose of 100 ergs/g and is applicable to any type of material and any type of radiation. Consider however, α-particles dissipating 1 rad as compared to β-particles dissipating 1 rad in biological tissue. Since the α-particles have a much higher LET, that is, they will produce more ionizations per unit track length, then the probability is higher that ionization will be produced in an important constituent of the cell. Thus, the relative biological effect of α-particles will be greater than β-particles. To account for this different relative effect of various particles, the absorbed dose in rads is multiplied by a weighting factor called QF (Quality Factor). The product of absorbed dose in rad and QF is termed dose in rem.

$$\text{Dose in rem} = \text{dose in rad} \times \text{QF}$$

One rem of α-radiation will then have the same biological effect as 1 rem of β-radiation. For general radiation protection purposes, the values of QF for various types of radiation are given in Table II.

The safe use of radionuclides and radiation can be divided into three categories: (1) protection of personnel, (2) control of contamination and (3) waste disposal.

TABLE II. QUALITY FACTORS (QF) FOR CONVERTING ABSORBED DOSE IN RAD TO DOSE IN REM

Radiation	QF
X- or γ-rays	1
β-particles E > 0.03 MeV	1
Thermal and slow neutrons	2.5
α-particles	10
Fast neutrons	10

3.2. Protection of personnel

The International Commission on Radiation Protection (ICRP) has recommended that the average yearly dose to radiation workers not exceed 5 rem/year. This is equivalent to 0.1 rem/week. (Reference [9] gives this recommendation in full detail.) This applies to whole-body dose from both internal and external radiation.

3.2.1. External exposure

Radiation dose to personnel must always be kept as low as possible and any unnecessary exposure must be avoided. In the case of external exposure, this can be accomplished by (1) sufficient shielding, (2) increasing working distance from the source and (3) minimizing exposure time. Shielding of α-emitters for external radiation is not required because the wall of the container or a few centimetres of air will absorb all particles. The same considerations generally apply to low-energy β-emitters such as ^{3}H, ^{14}C or ^{45}Ca. High-energy β-emitters require only 1 - 2 cm of low Z material, such as lucite, for shields. In the case of γ-rays, a high Z material, such as lead, provides the best shielding. Table III provides values of half-thicknesses of lead for shielding of γ-rays.

To obtain the approximate half-thickness of water, the corresponding half-thickness of lead may be multiplied by 10. (The density of lead is about ten times that of water.) To obtain the approximate half-thickness of any other material, the necessary half-thickness of water is divided by the density of that other material.

The attenuation factor F and the number of half-thicknesses n are related as follows

$$F = 2^{n}$$

or

$$n = \frac{\log F}{0.3} \tag{22}$$

Work with γ-ray sources should always be performed with sufficient shielding for personnel. The calculated dose rate after shielding should always be checked with a dose-rate meter, preferably an ionization chamber type. Sources not in use should always be stored behind shielding and access to the sources strictly controlled.

TABLE III. APPROXIMATE VALUE OF THE HALF-THICKNESS OF LEAD AS A FUNCTION OF GAMMA-RAY ENERGY

E_γ (MeV)	Approximate half-thickness of lead shielding (cm)
0.5	0.5
1.0	1
1.5	1.5
2-4	2

Radioactive materials; external dose rates less than 2.5 mrem/h	CAUTION RADIOACTIVE MATERIALS
External dose rates 2.5 ⟶ 100 mrem/h	CAUTION RADIATION AREA
External dose rates exceeding 100 mrem/h	CAUTION HIGH RADIATION AREA

It is of importance that before beginning any work with appreciable amounts of γ-ray emitters the researcher should know how great the γ-ray dose from the source will be. For point sources of activity, γ-ray intensity will decrease as the square of the distance (inverse-square law). Thus, the exposure dose can be calculated for any source as follows:

Exposure dose in R/h for A (Ci) at distance d (cm) from point γ-ray source

$$= \frac{A\ (\mathrm{Ci}) \times 2.22 \times 10^{12}\ (\mathrm{dis/min\text{-}Ci}) \times E\ (\mathrm{MeV/dis}) \times 1.6 \times 10^{-6}\ (\mathrm{ergs/MeV})}{60\ (\mathrm{min/h}) \times 4\pi d^2\ (\mathrm{cm}^2) \times 87.7\ (\mathrm{ergs/g\text{-}R}) \times 1.3 \times 10^{-3}}$$

$$\times \frac{\mu\ (\mathrm{cm}^{-1})}{(\mathrm{g/cm}^3)} \qquad (23)$$

where μ is the linear absorption coefficient in cm^{-1} for air of E energy γ-rays.

Equation (23) may be simplified if A is taken as 1 Ci, d as 100 cm (1 m) and μ as $3.3 \times 10^{-5}\ \mathrm{cm}^{-1}$ for all γ-rays in the range 0.1 - 3 MeV. Combining constants, Eq. (23) reduces to

$$\Gamma\ (\mathrm{R/h\text{-}Ci\ at\ 1\ m}) \simeq 0.5\ \overline{E} \qquad (24)$$

where $\overline{E}$ is the average γ-ray energy which can be evaluated from the decay scheme of that radionuclide and Γ is termed the γ-ray dose constant.

Once the exposure dose is known at any one distance, it may be calculated at any other distance by the inverse-square law.

The γ-ray dose constant Γ(R/h-Ci at 1 m for various radionuclides) is given in Table IV.

TABLE IV. GAMMA-RAY EXPOSURE DOSE LEVELS FOR SOME RADIONUCLIDES

Radionuclide	Predominant γ-ray energy (MeV)	Γ (R/h-Ci at 1 m)
^{60}Co	1.17 and 1.33	1.3
^{137}Cs	0.662	0.31
^{131}I	0.36	0.22
^{51}Cr	0.32	0.02
^{24}Na	2.76	1.84
^{226}Ra + daughters in equilibrium	Many different	0.83
^{42}K	1.52	0.14

The inverse-square law shows that distance is a very important factor in minimizing dose. Consider a source from which the γ-ray exposure dose was 1 mR/h at 10 cm. Any manipulations with the source by means of long forceps or tweezers would produce a negligible finger or whole-body dose. However, if the source were handled without tweezers, for instance with rubber gloves as the only protection, the radiation exposure dose at 1 mm distance would be 10 000 mR/h = 10 R/h to the finger tips.

The exposure time is equally as important in minimizing dose. Manipulations with sources should be performed rapidly but carefully.

Monitoring of external dose can be accomplished by the use of personnel dosimeters. These can be worn on the body or attached to the hands or wrists, if necessary, and provide an integrated dose over the total working period. Film badges or solid-state thermoluminescent dosimeters are the most common systems currently in use.

3.2.2. Internal exposure

The internal hazards of radionuclides involve some distinctly different considerations. Beta-emitters and particularly alpha-emitters become extremely hazardous on entry into the body. The protection against internal contamination largely involves prevention of accidental ingestion, inhalation or skin absorption of radionuclides. The International Commission on Radiological Protection has calculated maximum permissible body burdens of all the radionuclides and the maximum permissible concentrations in water and air that would produce such body burdens if chronic exposure conditions existed. The factors that determine the maximum permissible body burden of any radionuclide are:

(1) Particle radiation energy, LET and half-life
(2) Absorption from the gastrointestinal tract or lung tissue into body fluids
(3) Distribution into body organs
(4) Biological half-life, i.e. the time required for the body burden to decrease by one-half.

Considerations (2) and (3) also depend on the chemical and physical form of the radionuclide. Also, the solubility in body fluids will largely determine the absorption and transport of the radionuclide.

The recommended maximum permissible body burdens and corresponding maximum permissible concentrations in air and water are given in Appendix III.

3.3. Control of contamination

Contamination of laboratory, benches, glassware and operator by radionuclides must be avoided for two reasons:

(1) Laboratory contamination can result in internal exposure of the laboratory personnel and even be spread to areas where other personnel may be exposed.
(2) Experimental results may become doubtful.

A number of laboratory rules should, therefore, be strictly adhered to:

(1) Eating, drinking and smoking in the laboratory are strictly prohibited.
(2) Each person should wear a laboratory coat. This coat should be worn only in the laboratory space where the experiments with radionuclides are done, not in the counting rooms.
(3) When there is a risk that the hands may become contaminated, thin surgical gloves should be worn. These gloves have to be put on in such a way that the inside never touches the outside in order to prevent direct contamination of the skin. A detailed description of the procedure for putting on or removing gloves is given in Appendix IV.
As soon as the risk for contamination of the hands is no longer present, the gloves should be removed, as they constitute a source of contamination of glassware, equipment, faucet handles etc.
(4) Pipetting or the performance of any similar mouth suction is strictly prohibited. Syringes or propipettes must be used.
(5) Protective eye glasses or shields should always be worn in a radiochemistry laboratory. This will shield the lens of the eye from high-energy β-particles and will minimize eye injury in the event of a chemical accident.
(6) To prevent contamination of gloves, hands, or equipment, paper tissues should be handy and always used as an intermediate. After use, these tissues should be disposed of in foot-operated waste bins or large drums.
(7) All operations involving volatile materials, heating or digestion must be done under a hood. The air velocity at the hood face should be approximately 40 m/min.
(8) Any operation in which radioactive dust may arise should be carried out in a glove-box in which slightly negative pressure is maintained. In the exhaust system a dust filter must be present to collect radioactive particles. These precautions are particularly important in the case of alpha activity.

(9) All operations should be carried out over shallow trays. The bottoms of the trays should be covered with absorbent paper.
(10) Storage bottles should be available for dumping of liquid waste. These bottles should contain a small amount of ion-exchange resin to concentrate the activity.
(11) Avoid cross contamination by using glassware, can openers, tweezers etc. for one particluar radionuclide only.
(12) An end-window G-M survey meter should be available for contamination detection. In addition, it would be preferable to have an ionization-chamber survey meter for exposure dose measurements.
(13) Frequent surveys of laboratory work areas, equipment and personnel should be performed with the G-M survey meter to detect contamination. In the case of α-emitters, ^{3}H or electron-capture emitters, filter paper should be used to swab the suspected areas. The swabs should be counted with an appropriate detector.
(14) Before leaving the laboratory the hands, clothing and shoe soles should be checked with a suitable survey instrument or swabbed.

3.3.1. Decontamination

Decontamination of the skin should first be attempted with soft soap and water, possibly with a soft brush. Care should be taken to avoid damaging the skin by excessive washing. Often washing with a carrier solution will aid in removal through exchange with the radioactive isotope. Obviously, the carrier solution must be non-toxic to the skin.

Generally, the decontamination of glassware, metal surfaces or painted surfaces with radioactive material of high specific activity is greatly reduced by repeated washings with carrier solution. Stocks of carrier solution should therefore be present where contamination is likely to occur. A spreading agent may be very effective.

Material	Decontamination solution
Glass:	Either 10% nitric acid or 2% ammonium bifluoride or chromic acid or carrier in 10% hydrochloric acid.
Aluminium:	10% nitric acid, sodium metasilicate or sodium metaphosphate.
Steel:	Phosphoric acid plus a spreading agent.
Lead:	4$\underline{N}$ hydrochloric acid until a reaction starts, a dilute alkaline solution, followed by water.
Linoleum:	Xylol or trichlorethylene to remove wax surface.
Painted surfaces:	Spreading agent and ammonium citrate or ammonium bifluoride.

Wood and concrete:	Difficult to decontaminate. Partial or complete removal of the contaminated material will usually be the only effective method.

3.3.2. Special laboratory design features

A laboratory in which work with radioactive materials is done should have facilities that:

(1) minimize the incidence and spread of contamination;
(2) make possible rapid decontamination.

These facilities are further determined by the nature of the work which is going to be carried out. Three types of laboratories may accordingly be described (see Table V and Ref. [8]). Usually, an "A" laboratory will be associated with reactor operations or waste processing plants. For biological research "B" or "C" laboratories will generally be adequate.

A "C" laboratory may be any ordinary laboratory that has a good ventilating system and an exhaust hood. Floors and benches should have a surface which can be cleaned easily.

If larger quantities of radionuclides are to be used, for example for the dilution of stock solutions or the preparation of labelled compounds, then a "B" laboratory will be required.

The characteristics of a "B" laboratory may be listed as follows:

(1) The laboratory room should be separate from the counting rooms.
(2) Ventillation of the laboratory should be sufficient to exchange the total room volume 12 times per hour. The air flow should be from least active to most active areas. The fan for each hood should be at the top of the vent duct so there is negative pressure throughout the vent duct. The ventilation to the room should be separate from that to other rooms, particularly counting rooms. There should be a particulate filter in each exhaust duct.

TABLE V. FACILITIES FOR RADIOACTIVE MATERIALS WORK

Radiotoxicity of radionuclides (and examples of each)	Minimum significant toxic quantity	Type of laboratory or working place desired		
		Type C Good chemical laboratory	Type B Radionuclide laboratory	Type A High-level laboratory
Very high (^{90}Sr, ^{210}Po, etc.)	0.1 μCi	10 μCi or less	10 μCi - 10 mCi	10 mCi or more
High (^{45}Ca, ^{89}Sr, ^{131}I, etc.)	1.0 μCi	100 μCi or less	100 Ci - 100 mCi	100 mCi or more
Moderate (^{22}Na, ^{32}P, ^{35}S, ^{42}K, ^{60}Co, ^{82}Br, etc.)	10 μCi	1 mCi or less	1 mCi - 1 Ci	1 Ci or more
Slight (^{3}H, ^{14}C)	100 μCi	10 mCi or less	10 mCi - 10 Ci	10 Ci or more

(3) Shielded, lockable, separate storage areas should be available for radioactive sources.
(4) For facilitation of decontamination, floors and benches should be covered with vinyl or linoleum, preferably without seams. Under no circumstances should uncovered wooden or concrete floors and bench tops be allowed. Furniture should be of non-porous material.
(5) The G-M survey meter, the hand and foot monitoring station and laboratory coat hooks should be located just inside the entrance to the laboratory.
(6) Water faucets should be of a foot- or elbow-operated design for prevention of contamination.
(7) If possible, a shower for personnel decontamination should be located close to the laboratory.
(8) Drains should be located in the floor.
(9) Ridges and corners in which dust may accumulate and which are difficult to clean should be absent.

3.4. Waste disposal

Radioactive waste should be controlled and disposed of according to the recommendations of the ICRP (see Appendix V). Generally, liquid waste should be stored in polyethylene containers and not disposed to the sanitary sewer system through sinks. High-volume, low-activity liquid waste may be treated by ion-exchangers to reduce the volume. Solid waste should be put in foot-operated bins. All waste containers must have the appropriate label as well as a label stating the date and quantities of each radionuclide added.

If possible, it is advisable to store all liquid and solid waste until the activities present have peen reduced by radioactive decay such that it might be disposed of by usual methods. If this is not possible, as in the case of long-lived emitters, land burial may be necessary. In some countries a central organization is in charge of collection, storage and/or burial of radioactive materials.

Waste disposal can be a serious problem and if work with appreciable activity of long-lived radionuclides is anticipated, expert advice should be sought.

4. SOME UTILIZATIONS IN BASIC PRINCIPLE

By virtue of the very high sensitivity with which their radiations may be detected, radionuclides are widely used as indicators in tracer work whenever stable indicators are unobtainable or impractical. Many of the basic principles have been treated in the foregoing sections. However, the basic lecture matter includes additional sections which deal with some supplementary principles involved in pathway identification, tracer dilution, tracer kinetics, neutron activation analysis, neutron moderation applications and gamma-ray attenuation. The principal limitations involved in the use of radioactive tracers are discussed in the introduction to the "Applied Part" of this manual, and the experiments given in both parts are designed to illustrate a number of principles and applications.

5. THE TRACER METHOD

The tracer method is a technique to observe certain characteristics of a population of specific things such as molecules, organisms or other entities by observing the behaviour of the tracer. The substance to be traced is generally termed the tracee. The criteria for an ideal tracer is that it be indistinguishable from the tracee at the unit level and that the introduction of the tracer does not disturb the system. Both of these criteria are nearly perfectly met by radioactive isotopic tracers. Isotopes have identical chemical properties (only slight mass differences) and they can be obtained in very high specific activity. Therefore, the introduction of an isotopic tracer generally adds negligible mass to the system and does not disturb its kinetics.

5.1. Pathway identification

A most common use of a radioactive tracer is to follow the pathway of an entity in a chemical, physical or biological system. When the entity is, for example, an intact organism or an inorganic object, the radioactive label used may belong to any element. The choice of label will then be governed (1) by the ease of attachment or incorporation of the label and its stability, (2) by the ease of detection of its radiation and (3) by half-life, in order to be able to follow the label over the desired period of experimentation yet not unduly allow possible contamination of the experimental environment. When the substance to be traced is an organic material or compound, the radionuclide must belong to one of the elements in the compound. This often reduces the choice to ^{14}C or ^{3}H plus perhaps ^{32}P, ^{35}S, ^{36}Cl or ^{131}I. The label may be incorporated into the tracee through biological growth, chemical synthesis or exchange processes.

If the substance to be traced is a mineral nutrient, the label should be an isotope of that element. In general, elements in the same chemical group, e.g. alkali metals, have similar chemical properties but not sufficient for a radioisotope of one element to serve as the tracer for another element in the group.

If the tracer is introduced into the system, its identification in other parts of the system infers information about the possible pathways of that tracee in the system. A well-known example is the use of ^{14}C-labelled glucose to observe the pathways and intermediates in the glycolytic cycle. Another might be the migration of insects and identification of their predators in an ecology study.

5.2. Tracer dilution

The tracer dilution technique has been very useful in determination of the exchangeable mass of a substance in a system. The principle is: that for a given constant amount of tracer radioactivity, the final specific activity is inversely proportional to the exchangeable mass of tracee in the system.

The technique, introduced by Hevesy, is particularly useful when quantitative separations are not possible or are too tedious for the systems under study. In addition, it is the principal technique used to measure the exchangeable mass "in vivo".

Consider a system that contains an unknown amount, S grams, of a substance. To this system is added a known amount of a radioactive tracer of initial specific activity a_i, so that

$$a_i = \frac{R}{s} \tag{25}$$

where R = activity of the tracer in μCi (or counts/min)
s = mass of tracee associated with tracer.

If the tracer is allowed to mix in the system then, according to the dilution principle, the final specific activity, a_f, will be

$$a_f = \frac{R}{S + s} \tag{26}$$

substituting (25) into (26)

$$a_f = \frac{a_i s}{S + s} \tag{27}$$

and solving for S

$$S = s \frac{a_i}{a_f} - 1 \tag{28}$$

Therefore, to determine S, only the final specific activity need be measured, as a_i (and therefore s) is known. Quantitative separation of the tracee in the sample from the system is not necessary because the specific activity is independent of sample size, recovery, handling losses etc. However, it is a necessary condition that the tracer be completely mixed with the tracee in the system. This condition becomes very important in tracer dilution studies "in vivo".

Very commonly, s is negligible compared with S. This is the case with "carrier-free" or high specific activity tracers. By inspection of Eq. (26), if s is negligible with respect to S, then

$$S = \frac{R}{a_f} \tag{29}$$

and only the initial tracer activity and the final specific activity need be known.

A variation of the tracer dilution technique, called "inverse tracer dilution" enables determination of the amount of tracer in a system by the addition of a known amount of tracee. Let a_i be the initial specific activity in the system

$$a_i = \frac{R}{s} \tag{30}$$

Now R is the unknown. If an amount S of tracee is added and allowed to mix the final specific activity will be

$$a_f = \frac{R}{S + s} \tag{31}$$

Substituting (30) and (31) into (29) and solving for R

$$R = \frac{Sa_i}{\frac{a_i}{a_f} - 1} \tag{32}$$

Therefore, the determination of the specific activities before and after the mixing of S grams of tracee enables calculation of the total radioactivity of the system.

The dilution equations developed above apply as well to the use of non-radioactive tracers or indicators.

5.2.1. Two examples of tracer dilution

(1) Consider the problem of estimation of the volume of water in a vessel. This would be an example of a "closed system" since no water can enter or leave, that is, there is no communication with the external environment. If R μCi of tritiated water (^{3}HOH) of specific activity a_i (μCi/ml) is pipetted into the vessel and allowed to mix, then the water volume of the vessel in millilitres can be calculated directly by Eq. (26)[2].

(2) Consider as a second example the estimation of the volume of water in an animal. This would be an example of an "open system", presumably in the steady state with respect to water exchange with the external environment. In the steady state the intake rate is equal to the outgoing rate and the exchangeable mass is constant. If R μCi of very high specific activity tritiated-water tracer is injected into the animal, then the water volume (total body water) can be calculated using Eq. (29). The specific activity a_f (μCi/ml) is determined by sampling plasma or urine after mixing is complete. However, the animal will have lost some fraction of the initial amount R via excretion during this mixing period. Therefore, the total activity excreted during the mixing must be collected and subtracted from R. Equation (29) is modified to

$$S\ (\mathrm{ml}) = \frac{R - R_{ex}\ (\mu\mathrm{Ci})}{a_f\ (\mu\mathrm{Ci/ml})} \tag{33}$$

where R_{ex} = μCi of tritiated water excreted via all routes up to the time the sample containing a_f is taken.

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[2] Although specific activity is expressed in these two examples as μCi/ml of water, it is actually the mass of exchangeable hydrogen being determined.

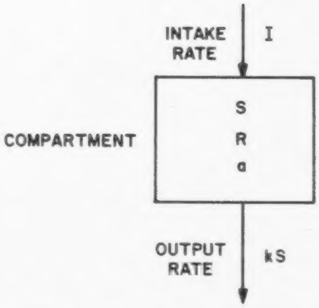

FIG. 12. Schematic model of single open compartment.
S = Tracee in grams
R = Radioactivity of tracer in μCi
a = Specific activity

$$= \frac{R}{S} \frac{\mu\text{Ci}}{\text{gram}}$$

I = Intake rate in grams of S per unit time.

5.3. Tracer kinetics

The principal difficulty in the tracer dilution technique is to ensure uniformity of mixing of the tracer. To determine the mixing time it is necessary to take repetitive samples as a function of time from the system. Such data contain valuable information on the kinetics of the mixing processes. It is such analysis as well as the response of the system at "tracer equilibrium" that is treated by the field of tracer kinetics.

Most biological systems are open, that is, there is exchange with their environment. Consider an open compartment as shown in Fig. 12 in the steady state. A compartment is a subdivision of a system in which the tracer specific activity is constant within its boundaries at any given time. Thus, the tracer specific activity defines the boundaries of a compartment and they may or may not coincide with any chemical, physical or physiological boundaries. Mixing within a compartment is rapid compared with the rate that tracer leaves the compartment.

If the compartment is in the steady state then the input rate is equal to the output rate or

$$I = kS \tag{34}$$

Therefore, S is constant but the tracer activity R and the specific activity a vary with time. (See caption to Fig. 12 for the symbol definitions.)

Consider now a compartment in which a tracer has been injected and allowed to mix. Most compartments in nature are observed to follow first-order kinetics. That is, the specific activity of the tracer is observed to decline exponentially, as

$$a = a_0 e^{-kt} \tag{35}$$

where k is the first-order rate constant.

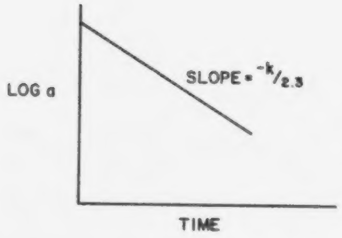

FIG. 13. Specific activity versus time in a single compartment after mixing of the tracer.

A plot of specific activity (after mixing) versus time would appear as in Fig. 13.

The slope of the line allows calculation of k. Exactly analogous to radioactive decay, a half-life may be determined graphically or by

$$T_{\frac{1}{2}B} = \frac{0.693}{k} \tag{36}$$

$T_{\frac{1}{2}B}$ is commonly termed the biological half-life. Since it was assumed that the tracer behaved exactly as the tracee it is now possible to calculate the output rate kS. S can be determined by tracer dilution.

As can be inferred from Eq. (34) the biological half-life is not a true biological constant but inversely related to the intake. If the intake rate increases by a factor of two, the biological half-life decreases by a factor of two, etc.

If the tracer undergoes significant radioactive decay during the experiment, the observations as plotted in Fig. 13 must be corrected for this radioactive decay. If this is not done, an "effective half-life" will be observed, accounting for both radioactive decay and biological loss. Since these processes are independent,

$$\lambda_{\text{eff}} = \lambda + k \tag{37}$$

where λ = radioactive decay constant of tracer

k = biological rate constant

The effective half-life then is:

$$T_{\frac{1}{2}\text{eff}} = \frac{0.693}{\lambda_{\text{eff}}} \tag{38}$$

Consider now the case when tracer is supplied to the compartment at a constant rate i (μCi per unit time). Since a constant fraction of the tracee will also be lost per unit time, the specific activity in the compartment will increase from zero to a maximum value. The differential equation describing this rate of change is given by

$$\frac{da}{dt} = \text{intake rate minus loss rate} = i - ka \qquad (39)$$

Since a increases with time the loss rate will increase until it is equal to the intake rate and $da/dt = 0$. The compartment would then be in the tracer steady state. Equation (39) can be integrated to give

$$a = \frac{i}{k}(1 - e^{-kt}) \qquad (40)$$

or

$$ka = i\,(1 - e^{-kt}) \qquad (41)$$

In this case, a plot of a versus time will be as shown in Fig. 14. The specific activity will reach $\frac{1}{2}$ of the steady-state value a_∞ in a time equal to $T_{\frac{1}{2}B}$, $\frac{3}{4}$ of this value in $2T_{\frac{1}{2}B}$ etc.

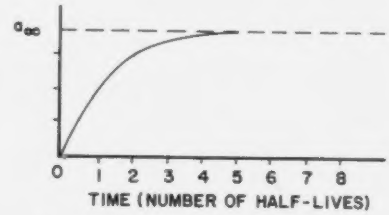

FIG. 14. Specific activity in a single compartment following initiation of constant intake rate.

The behaviour of tracee in such steady-state systems is commonly termed turnover. The turnover time, i.e. the average time a tracer atom or molecule spends in the compartment is given by

turnover time or average life time $\bar{t} = \frac{1}{k}$ (42)

Since $k = 0.693/T_{\frac{1}{2}B}$

then $\bar{t} = 1.44\,T_{\frac{1}{2}B}$ (43)

The derivation of Eq. (42) is given in Appendix VI.

It should be apparent that once the tracer has mixed completely in the whole system, though it probably will be composed of many compartments, its behaviour will be as in a single compartment. Some description of a three-compartment open model is given in Appendix VII.

The general equations for a closed two-compartment model are not unduly rigorous and will be presented below. Consider the model as shown in Fig. 15.

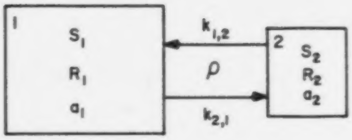

FIG. 15. Closed two-compartment model in steady state.

$k_{1,2}$ = first-order rate constant describing exchange of tracee or tracer from compartment 2 to compartment 1
$k_{2,1}$ = first-order rate constant describing exchange of tracee or tracer from compartment 1 to compartment 2
ρ = rate at which tracee is exchanged, i.e. grams/unit time

If compartment 1 is initially labelled, the following differential equations may be written

$$\frac{dR_1}{dt} = \frac{S_1 da_1}{dt} = \rho\,(a_2 - a_1) \tag{44}$$

$$\frac{dR_2}{dt} = \frac{S_2 da_2}{dt} = \rho\,(a_1 - a_2) \tag{45}$$

Let $\Delta_{1,2}$ equal the difference in specific activities at any time.

$$\Delta_{1,2} = a_1 - a_2 \tag{46}$$

$$da_1 - da_2 = d\Delta_{1,2} = -\rho\left(\frac{1}{S_1} + \frac{1}{S_2}\right)\Delta_{1,2} \tag{47}$$

Equation (47) is observed to be a first-order differential equation identical in form to Eq.(1) and may be integrated directly to give

$$\Delta_{1,2} = a_1(0)\, e^{-\rho\left(\frac{1}{S_1} + \frac{1}{S_2}\right) t} \tag{48}$$

where $a_1(0)$ is the initial condition that at $t = 0$ all the activity is in compartment 1. Therefore, $\Delta_{1,2}(0) = a_1(0)$.

Now since the system is closed, the total activity is constant. This can be expressed as

$$S_1 a_1 + S_2 a_2 = S_1 a_1(0) \tag{49}$$

If Eqs (48) and (49) are solved simultaneously, the following solutions are obtained

$$a_1 = \frac{a_1(0)}{S}\left(S_1 + S_2 e^{-\rho St/S_1 S_2}\right) \qquad (50)$$

$$a_2 = \frac{a_1(0)\,S_1}{S}\left(1 - e^{-\rho St/S_1 S_2}\right) \qquad (51)$$

where $S = S_1 + S_2$

Plots of a_1 and a_2 versus time are shown in Fig. 16.

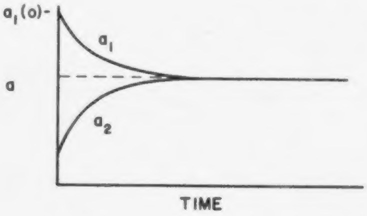

FIG. 16. Specific activity in a closed two-compartment system with compartment 1 initially labelled.

Total S may be determined by the dilution technique. Now by inspection of Eq. (50) at long times, i.e. when the tracer is completely mixed, a_1 equals the equilibrium value

$$a_1 = \frac{a_1(0)\,S_1}{S} \qquad (52)$$

Therefore, one can solve for S_1 and hence S_2 by difference. Now a plot of Eq. (48) will allow calculation of the slope

$\rho\left(\frac{1}{S_1} + \frac{1}{S_2}\right)/2.3$ (see Fig. 17).

Therefore, from the slope, the transfer rate ρ may be determined. To obtain transfer rates between compartments is the objective of most tracer kinetic experimentation. It should be noted that

$$\rho = k_{1,2}S_2 = k_{2,1}S_1 \qquad (53)$$

which expresses the steady-state condition.

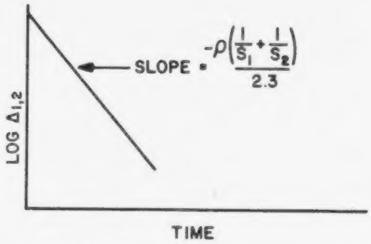

FIG. 17. Plot of Eq. (48) for closed two-compartment model.

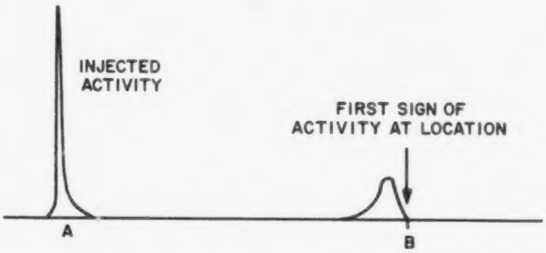

FIG. 18. Illustration of diffuse appearance of tracer at location B following bolus injection at location A.

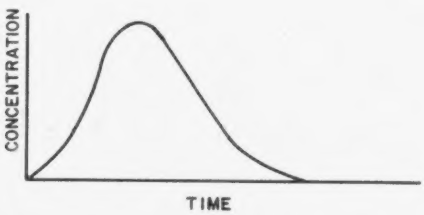

FIG. 19. Schematic curve of tracer concentration at location B following single bolus injection at location A.

A final example of the use of tracers can be termed translocation or rate of flow studies. If a radioactive tracer is injected at one location (A) in a system and its appearance observed at another location (B), the shape of the injection pulse will have become diffuse due to the number of possible pathways between locations A and B. See Fig. 18.

If a known amount of label R is injected at location A and the concentration of label appearing at B is measured as a function of time, then a typical curve as shown in Fig. 19 may result.

If no tracer is lost between A and B (only a labyrinth in between) and if the area under the curve in Fig. 19 can be determined (either graphically or by integration) then the flow rate in the system, Q, can be calculated from the Stewart-Hamilton principle.

$$Q\,\frac{(\text{ml})}{(\text{time})} = \frac{R}{\text{area under curve}}\quad\frac{(\mu\text{Ci})}{(\mu\text{Ci/ml})\,(\text{time})} \tag{54}$$

Calculation of flow rate by this method has been very useful in physiological circulation studies and can be adapted to natural systems easily.

6. NEUTRON ACTIVATION ANALYSIS

If a stable element is exposed to a flux of neutrons there is a finite probability that a stable nucleus can capture a neutron to produce the isotope of that element with an increase in mass number of 1. As discussed in Section 1.4.4 this activation process is a primary method of producing artificial radionuclides. An example of such a capture reaction is

$$^{23}_{11}\text{Na} + {}^{1}_{0}\text{n} \longrightarrow {}^{24}_{11}\text{Na} + \gamma$$

In general such activation reactions are most probable with thermal or slow neutrons. The activation reaction is utilized in an analytical technique termed neutron activation analysis, as follows.

Let n_T be the number of stable nuclei exposed to a flux Φ of thermal neutrons. Let σ represent the cross-section per nucleus or the probability of a capture reaction occurring. Therefore, the rate of production of radioactive atoms N will be

$$\frac{dN}{dt} = \sigma n_T \Phi \tag{55}$$

where σ = cross-section in units of cm^2/nucleus
n_T = total number of exposed stable nuclei
Φ = thermal neutron-flux in neutrons/cm sec.

However, the radioactive atoms, N, produced by activation will immediately begin to decay at their own rate characterized by the $T_{1/2}$ of the radioactive isotope produced. Therefore Eq. (55) must be modified to include this rate of decay

$$\frac{dN}{dt} = \sigma n_T \Phi - \lambda N \tag{56}$$

$$\text{net rate} = \begin{pmatrix}\text{rate of}\\ \text{production}\end{pmatrix} - \begin{pmatrix}\text{rate of radio-}\\ \text{active decay}\end{pmatrix}$$

Equation (56), similar to Eq. (39), is integrated to the following

$$A = N\lambda = \sigma n_T \Phi\,(1 - e^{-\lambda t}) \tag{57}$$

where A = activity produced in dis/sec
λ = decay constant of radioisotope produced
t = time of irradiation

Most commonly the technique is used to determine the number of atoms n_T of the stable element in a sample. The flux Φ, irradiation time t, and cross-section σ are known and the activity produced is measured allowing measurement of n_T. A comparator technique, i.e. irradiating a sample with a known amount of the element, is frequently adopted.

Using nuclear reactors as the source of neutrons the technique is extremely sensitive for certain elements which cannot be analysed by conventional chemical methods.

As seen from Eq.(57) the element for analysis should have high abundance of the stable isotope, a high cross-section σ, and a half-life that is not so short to preclude easy measurement or so long that long irradiation times are necessary. The detection limits for certain elements can be as low as 10^{-12} grams.

7. NEUTRON MODERATION AND GAMMA-RAY ATTENUATION TECHNIQUES

7.1. Neutron moderation in relation to moisture determination

Neutrons can be produced in the laboratory by using small sources containing α-particle emitters and finely divided beryllium. The α-emitters are commonly either ^{239}Pu or ^{241}Am. Both have long half-lives and emit negligible γ-rays. The nuclear reaction responsible for the neutron production is

$$^{4}_{2}\mathrm{He} + ^{9}_{4}\mathrm{Be} \longrightarrow ^{12}_{6}\mathrm{C} + ^{1}_{0}\mathrm{n}$$

The neutrons produced from such a source have an average kinetic energy of approximately 4.5 MeV, but the energies range from 0-12 MeV.

As discussed in Section 1.4.4, moderation or slowing down of fast neutrons by elastic collisions is most efficient with small atoms, in particular hydrogen. Water is a very efficient moderator (although some neutrons are lost by (n, γ) capture by ^{1}H). The BF_3 tube (discussed in Section 2.6) used to detect neutrons is sensitive only to slow or thermal neutrons. Thus, if a BF_3 counter tube is placed near a fast-neutron source, the count rate in the tube will be directly proportional to the amount of moderation between the source and the detector. Soil scientists have used this principle to measure the water content of soils in situ. Most hydrogen atoms in soil are in molecules of water. Therefore, the count rate of a BF_3 tube in a soil well containing also a neutron source is directly proportional to the moisture content of the soil near the detector and source. The system can be calibrated either by taking soil core samples near each reading and determining their water content by oven drying, or by taking a series of readings in soil barrels in which the water content is controlled.

Sources of error in the technique are principally due to elements that have a large thermal neutron absorption cross-section (e.g. Cd, B, Mn, Cl and Li). Fortunately, these elements are generally present in such small concentrations that they produce little effect. However, in some soils this source of error must be considered.

7.2. Gamma-ray attenuation in relation to density determination

As discussed in Section 1.4.3, the interaction of γ-ray photons with matter is largely with orbital electrons. For medium and low Z materials, the predominant effect is Compton scattering and absorption. The Compton effect is proportional to the Z/M ratio or electrons/gram in the attenuating materials. For most low and medium Z elements, the Z/M ratio is very nearly 1/2. Thus, if the Z/M ratios are nearly the same, the attenuation of γ-rays will be proportional to the bulk density or number of electrons/gram. The bulk density of medium and low Z materials, such as soil, can be determined by this principle. Usually a ^{137}Cs γ-ray source is used and the count rate of a NaI(Tl) scintillation-counter detector will be inversely proportional to the bulk density in the material between the source and the detector.

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LABORATORY EXERCISES

1. EXPERIMENTS WITH A G-M COUNTER

1.1. The plateau of a G-M tube

INTRODUCTION

Geiger-Müller counter assemblies in normal operation often show an appreciable variation in performance from time to time. It is thus useful to have a reference source by which day-to-day counting may be standardized. The half-life of such a standard should be so long that no correction for decay need be made. A suitable reference source may be made from black uranium oxide (U_3O_8). This combines the required chemical stability and long half-life (4.5×10^9 yr). The oxide should not have been treated chemically for at least a year, during which time any significant daughter products removed by previous treatments will have again come to radioactive equilibrium.

The total disintegration scheme of ^{238}U and daughters is very complex, and it is advisable to filter out all particles except the β-particles of 2.3 and 1.5 MeV from ^{234m}Pa. This can be done by covering the source with aluminium foil of approximately 35 mg/cm^2 thickness. If this is done the U_3O_8 source may be used as an absolute standard. Pure U_3O_8 undergoes 724 dis/min per mg. The 2.3-MeV β-particle is given off in greater than 99% of the disintegrations.

With this or a similar standard beta reference source the following properties of a G-M tube may be determined:

(1) The starting potential and threshold voltage.
(2) The characteristic curve of count rate versus tube voltage and the counting plateau.
(3) The optimum operating voltage.
(4) The counting yield Y (counts/dis).

EXPERIMENTAL PROCEDURE

(1) Obtain a source counting about 5000 counts/min. If the source is U_3O_8, weigh the source.
(2) Put the source (on a planchet) into the planchet holder in the shield. Be sure the high-voltage switch is off and turned to its minimum position. Turn on the master power switch and the instrument to "count" mode. Now turn on the high-voltage switch.
(3) Increase the voltage slowly until the first counts are obtained. The voltage is called the starting potential.
(4) Determine the count rate with increasing voltage. A total of 2000 counts for each voltage step is adequate. Increase the voltage in steps of 25 or 50 volts.
(5) When the count rate does not change appreciably as the voltage is increased the threshold voltage has been reached and the G-M tube is operating in the plateau region. When the count rate begins to increase rapidly no further high-voltage steps should

be applied. This is termed continuous discharge and above this voltage the counter will race and damage to the G-M tube will occur.

(6) Calculate the slope as the percentage increase in count rate per 100 volts and the plateau length $V_2 - V_1$ (see Fig. 20). The slope is

$$\frac{100\ (r_2 - r_1)r_1}{V_2 - V_1} \times 100 = \%\ \text{per volts} \qquad (1)$$

A good G-M tube will have a slope of less than 10% per 100 volts.

(7) As the tube ages the plateau length will begin to decrease. To allow for this choose an operating voltage of $V_1 + 75$ volts, or $\frac{1}{2}(V_1 + V_2)$ if the plateau is less than 150 volts. Occasional checks on the characteristic curve should be performed as the tube ages. (Age is proportional to the total number of counts).

(8) At the operating voltage, if the disintegration rate of the standard source is known, calculate the counting yield Y at each shelf position in the shield.

$$Y = \frac{r\ (\text{counts/min})}{A\ \text{dis/min}} \qquad (2)$$

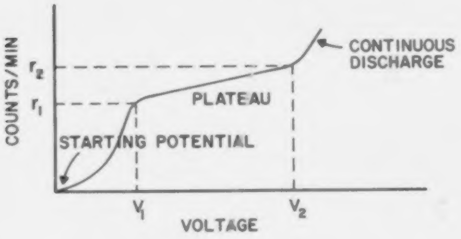

FIG. 20. Characteristic curve for Geiger-Müller tube.

1.2. The resolving-time of a G-M counter

INTRODUCTION

The resolving time, i.e. the time after each pulse that the G-M tube is not able to register pulses, can be determined in various ways. The method by which a series of samples of increasing strength is counted is straightforward. From the difference between the expected count rate as extrapolated from low counting rates and the observed count rate, the resolving time can be estimated.

As shown in Section 2.2, if the resolving time τ is known then the true count rate R can be calculated from the observed count rate r as follows

$$R = \frac{r}{1 - r\tau} \qquad (1)$$

If the r of a radionuclide of known half-life is plotted against time on semi-log paper, R of the highest count rates can be extrapolated from the r of the lowest counting rates and τ can then be estimated by use of Eq. (1).

Another approximation of the resolving time τ may be obtained by the method of "twin samples", i.e. from a comparison of the count rates of each sample counted separately.

Let R_1, R_2, $R_{1,2}$ and R be the true count rates (background included) of sample 1, sample 2, samples 1 plus 2, and a blank sample, respectively. Also let r_1, r_2, $r_{1,2}$ and r be the corresponding observed count rates. Then by definition

$$R_1 + R_2 = R_{1,2} + R \tag{2}$$

Substituting in Eq. (1)

$$\frac{r_1}{1 - r_1\tau} + \frac{r_2}{1 - r_2\tau} = \frac{r_{1,2}}{1 - r_{1,2}} + \frac{r}{1 - r\tau} \tag{3}$$

Since $r_1\tau \ll 1$ and $r\tau \ll r_1\tau$, the following approximations can be made

$$\frac{r_1}{1 - r_1\tau} \cong r_1 + r_1^2\tau \quad \text{and,} \quad \frac{r}{1 + r\tau} \cong r$$

Therefore, after substituting, we obtain

$$\tau = \frac{r_1 + r_2 - r_{1,2} - r}{r_{1,2}^2 - r_1^2 - r_2^2} \tag{4}$$

which can be simplified to

$$\tau \cong \frac{r_1 + r_2 - r_{1,2} - r}{2r_1 r_2} \tag{5}$$

since

$$r_{1,2}^2 \cong (r_1 + r_2)^2$$

PROCEDURE

(1) Tap about 5 ml (approx. 25 000 counts/min) from the ^{137m}Ba column used in Experiment 2.2; cover the counting dish with a plastic cap of 100 - 150 mg/cm^2 and immediately start making 6 - 8 one-minute countings separated by one-minute intervals. Very accurately record the elapsed time.

(2) After half an hour determine the residual count rate (background) then plot the count rates corrected for background of ^{137m}Ba versus time on semi-log graph paper.

(3) A straight line with a slope corresponding to the "tenth-life" of ^{137m}Ba (8.5 min) is drawn through the last 3 or 4 points. Now from the small deviations of the first few points from this straight line, estimate the resolving time of the G-M counter using Eq. (1). Note: Verify that $T_{\frac{1}{2}} \times 3.33 = T_{\frac{1}{10}}$.

(4) Select two samples of β-emitting radionuclides (e.g. ^{204}Tl) of approximately 12 000 counts/min each.
(5) Count the first sample in a sample holder having two holes. In the second hole insert a blank planchet.
(6) After counting the first sample, remove the blank planchet and replace it with the second sample. Now count both samples together.
(7) Remove the first sample and replace by a blank planchet and count the second sample.
(8) Determine the background by counting with two blank planchets in counting position.
(9) Calculate τ by use of Eq.(5).
(10) Use an end-window G-M tube survey meter to survey for personnel or equipment contamination.

1.3. Counting and sampling statistics

In scientific experimentation the standard deviation (calculated from replicates) should always be given together with the results to permit assessment of the uncertainty. When the standard deviation is calculated from replicate analyses it automatically includes all sources of variation, i.e. those due to the experimental methods as well as the natural variation inherent in radioactive decay.

If a total of C counts are obtained on a single sample, the standard deviation of that single count is estimated by

$$\sigma = \sqrt{C} \tag{1}$$

If the sample is counted repeatedly without moving the sample and assuming the counter system performs perfectly, the standard deviation of that series of counts, as calculated by statistics of the normal distribution, will give essentially the same estimate. The standard deviation will only include the variation due to radioactive decay which we will term natural uncertainty.

However, if the sample is moved between countings or a number of "identical" samples are counted in succession, a larger value will generally be obtained if the standard deviation is calculated by the latter method. This is due to random variations in geometry and sample preparation. These additional sources of variation including erratic counter performance we will term technical uncertainty. An experimental evaluation of these two types of uncertainty will be made.

MATERIALS AND REAGENTS

(1) ^{32}P solution of approximately 0.1 μCi/ml.
(2) 0.11-ml pipette and pro-pipette (e.g. rubber bulb or syringe).
(3) 25 counting planchets.
(4) Infra-red drying lamp.

PROCEDURE

(1) Using the pro-pipette, pipette 25 samples containing 1 ml each. Dry each sample under an infra-red lamp. Do not allow the samples to boil or spatter.

(2) Place one of the dry samples in the planchet holder and make 25 countings of 1 min each without moving the sample. Record the result of each counting.

(3) Calculate the natural standard deviation as

$$\sigma_{nat} = \sqrt{\bar{C}}$$

where $\bar{C}$ is the mean value of the 25 countings.

(4) Calculate the total standard deviation by the following equation

$$\sigma_{tot} = \sqrt{\frac{\sum (C - \bar{C})^2}{n - 1}} \qquad (2)$$

where C = count of each individual counting
$\bar{C}$ = mean value of the 25 countings
n = number of countings ($n = 25$ in this case)

(5) Compare σ_{nat} and σ_{tot}, and if they are found to be significantly different, explain. Calculate σ_{nat} in per cent.

(6) Now count each of the 25 samples separately for 2 min, and record the results.

(7) Repeat the calculation of σ_{tot} according to Eq. (2), and compare with σ_{nat}. Calculate σ_{tech} in per cent using the equation

$$\sigma_{tot}^2 = \sigma_{nat}^2 + \sigma_{tech}^2 \qquad (3)$$

(8) Use the end-window G-M survey meter to survey for personnel or equipment contamination.

1.4. Absorption of beta particles

The absorption of β-particles in matter is very nearly independent of the atomic number of the absorbing material provided the thickness is expressed in mg/cm^2 (thickness × density). Beta particles have a spectrum of energies ranging from zero to maximum value for each particular radionuclide. ^{32}P emits only β-particles. The maximum energy is 1.7 MeV and the average energy 0.7 MeV. The thickness of matter which is able to absorb all incident β-particles is called the "range", and this is determined by the maximum energy particles. However, only a small fraction of the beta particles from any source have this maximum energy and the range is therefore not sharply defined. For ^{32}P the range is approximately 800 mg/cm^2.

A transmission curve of ^{32}P beta particles through aluminium will be prepared in the present experiment. A simplified method to determine β-particle range and energy is also demonstrated.

PROCEDURE

(1) Obtain a ^{32}P solution of approximately 0.1 μCi/ml.

(2) Pipette 100 λ (0.1 ml)[3] onto a planchet and dry under an infra-red lamp.

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[3] 1 λ = 1 μ-litre

(3) Prepare a second sample by pipetting 100 λ of a ^{32}P solution of approximately 50 μCi/ml into a planchet and drying.

(4) Count the low-activity source in the G-M counter. Obtain at least 10 000 counts. Place an aluminium filter of about 20 mg/cm^2 between the counter window and the source and count again.

(5) Continue counting at increasing absorber thickness until a count rate of about 200 counts/min is obtained.

(6) Repeat the previous two counts with the high-activity source and calculate the average ratio between low- and high-activity source count rates (Note: resolving time corrections must be made for the high-activity source counts and background must be subtracted in the case of the low-activity source counts). This ratio can be used to transform the low-activity source count rates into the high-activity source count rates. The high-activity source cannot be counted at zero or low absorber thicknesses.

(7) Continue counting the high-activity source with increasing absorber thickness until an almost constant count rate is obtained. The count rate now is due to bremsstrahlung or continuous X-rays produced by interaction of the β-particles with the Al nuclei.

(8) Plot the observed and calculated net count rates of the high-activity source on the log co-ordinate versus absorber thickness on the linear co-ordinate. (To the absorber thickness add the window thickness of the G-M tube and the air thickness, in mg/cm^2, from G-M tube window to source.)

(9) Extrapolate the bremsstrahlung component to zero absorber thickness and subtract this contribution from the net count rate. Plot the corrected curve.

(10) Determine by inspection the point at which the uncorrected transmission curve appears to intersect the bremsstrahlung curve. This point corresponds to the range of ^{32}P β-particles and should be approximately 800 mg/cm^2.

(11) A simplified method of determining β-particle range is based on determination of the fifth half-thickness of absorber. The half-thickness is the thickness of absorber required to reduce the count rate by a factor of 2. The fifth half-thickness is the amount required to reduce the count rate by a factor of 25 or 32. The fifth half-thickness has been found empirically, in most cases, to be approximately equal to $\frac{1}{2}$ the range of the maximum-energy β-particle.

From the corrected curve, determine the thickness of absorber that was required to reduce the count rate at zero absorber by a factor of 32. Multiply this value by 2 and determine how well it estimates the range of ^{32}P particles.

(12) Repeat the exercise for ^{14}C instead of ^{32}P. The "range" for ^{14}C should be about 30 mg/cm^2.

(13) How well can the initial portions of each corrected curve approximate a straight line?

(14) Use the end-window G-M survey meter to survey for personnel or equipment contamination.

1.5. Self-absorption and self-scattering of beta particles

INTRODUCTION

It is often necessary to measure the radioactivity of sources which contain appreciable amounts of solid material. When a thick source is counted, errors from self-absorption and from source scattering are introduced. Self-absorption decreases the count rate below the expected value and is most important with low-energy β-emitters whose maximum energy is less than 0.5 MeV. Scattering tends to increase the count rate and is most noticeable with high-energy β-emitters. (The effect of self-absorption and self-scattering also exists with γ-ray emitters but is usually negligible since γ-radiation has a greater penetrating power.) A third source of error when voluminous samples of varying thickness are involved may be called "self-geometry", i.e. the top of the sample becomes closer to the counter as the sample thickness increases. The combined effects of self-absorption, self-scattering and self-geometry, normally result in a decrease in the count rate. The principal method commonly used to correct the self-absorption is as follows:

The method is based on the assumption that the absorption of beta particles is an exponential function of absorber thickness. (To verify this, observe that in the previous experiment the absorption was closely approximated by an exponential function over the first decade or two.) Therefore, assume that the decrease in count rate per unit sample thickness is proportional to the sample thickness or

$$\frac{dm}{dx} = -\mu m \tag{1}$$

where m = count rate per cm^3 thickness of sample
μ = linear absorption coefficient of the self-absorbing material in cm^{-1}
x = sample thickness in cm

Equation (1) can be integrated to

$$m = m_0 e^{-\mu x} \tag{2}$$

where m_0 = count rate per cm^3 of those atoms located on the surface of the sample
m = count rate per cm^3 at thickness x of sample

The total observed count rate then from a sample of cross-sectional area θ (cm^2) and thickness X is obtained by integrating the activity as a function of thickness over the entire thickness of the sample, X.

$$r = \int_0^X \theta m_0 e^{-\mu x}\, dx = \frac{m_0 \theta}{\mu}(1 - e^{-\mu X}) \tag{3}$$

However,

$$m_0 \theta = \frac{R}{X}$$

where R = count rate if there is no self-absorption

Therefore

$$r = \frac{R}{\mu X}(1 - e^{-\mu x}) \qquad (4)$$

Finally, defining a self-absorption factor f as the ratio between observed count rate and the count rate if there were no self-absorption, as

$$f = \frac{r}{R} = \frac{1}{\mu X}(1 - e^{-\mu x}) \qquad (5)$$

Therefore, for samples of X thickness, the factor f may be used to correct observed count rates for self absorption. The constant must be obtained experimentally.

If μ is expressed as the mass absorption coefficient (μ/ρ, cm^2/g) and the thickness as density-thickness ($x\rho$, g/cm^2) then μ will be largely independent of the absorber material and only a function of the maximum energy of the β-particles.

As in Fig. 11, Section 2.4 of the Lecture Matter, if samples of constant specific activity but increasing thickness are counted the count rate will asymptotically approach a constant value. Samples of this thickness or greater are said to be infinitely thick and if a standard of known specific activity is available, then unknown samples at infinite thickness may be determined by simple proportion.

$$\frac{r_{\text{unknown}}}{a_{\text{unknown}}} = \frac{r_{\text{known}}}{a_{\text{known}}} \qquad (6)$$

where r = count rate
a = specific activity

If it is impossible to prepare infinitely thick samples, the following adaptation may be used.

If the data for Fig. 11, Section 2.4, is replotted as count rate per unit sample weight versus sample weight, a curve as in Fig. 21 will result.

The curve is extrapolated to zero mass to obtain the estimate of the "true" counts/min mg. Now this is assigned the value 1 and the value at each sample mass is calculated as a fraction of the "true" value. These fractions are plotted against sample mass to give a calibration curve for self-absorption in samples of intermediate thickness. See Fig. 22.

Thus we can determine the total activity of a sample of unknown concentration and intermediate thickness by:

(1) Weighing and counting the sample. Determine apparent specific ability.

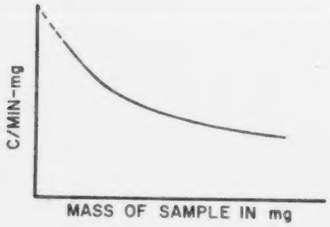

FIG. 21. Mass of sample in mg.

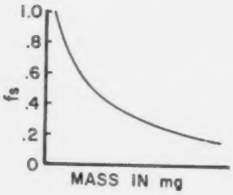

FIG. 22. Mass in mg.

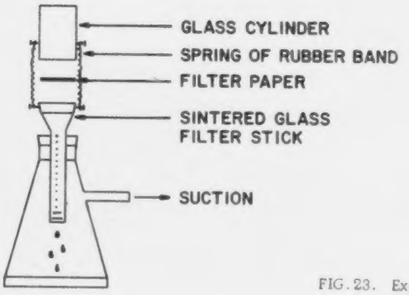

FIG. 23. Exploded view of filtering assembly.

(2) Determine from a graph, as in Fig. 22, the fraction of its activity that will be counted.
(3) Divide the apparent specific activity by the above fraction to obtain specific activity.
(4) Total activity = mass of sample × specific activity.

The following experiment will demonstrate the above techniques.

MATERIAL AND REAGENTS

Eight 1-in. planchets
Filter apparatus
Eight 1-in. glass filter papers
50-λ, 100-λ, 150-λ, 250-λ, 400-λ, 500-λ, 1-ml and 2-ml pipettes
Solution of $Na_2^{14}CO_3$ (approximately 3 mg/ml and 0.05 μCi/ml)
$BaCl_2$ solution.

PROCEDURE

(1) Place the following measured aliquots of a known stock solution of radioactive sodium carbonate in six centrifuge tubes: 50 λ, 100 λ, 150 λ, 400 λ, 500 λ, 1 ml, 2 ml and 5 ml.
(2) Precipitate the radioactive carbonate by the addition of excess $BaCl_2$ solution.
(3) Filter samples on preweighed 1-in. filters and wash three times with 5 ml of distilled water (see Fig. 23). Air dry. Fix precipitate with 1 ml of collodian solution to prevent spread of contamination. Be sure samples are thoroughly dry.
(4) Weigh samples. Place in planchets and count.
(5) Count background with an empty planchet in counter.
(6) Prepare curves from background-corrected counting data as described in the introductory material; i.e. count rate vs. sample mass, count rate/mg vs. sample mass, and fraction of true count rate vs. sample mass.
(7) Calculate the value of μ. For ^{14}C it should be approximately $0.28\ cm^2/mg$. μ can be determined from any point at infinite thickness since at infinite thickness x becomes large, therefore $e^{-\mu x} \longrightarrow 0$, $1 - e^{-\mu x} \longrightarrow 1$.
(8) Use the end-window G-M survey meter to survey for personnel or equipment contamination.

2. EXPERIMENTS WITH A SCINTILLATION COUNTER

2.1. Solid scintillation counting

The following solid scintillation crystals are used to detect radiation:

α-particles	ZnS (activated by silver) spread as a thin layer 10-20 mg/cm^2
β-particles	Anthracene, trans-stilbene
γ-rays	NaI (activated by thallium at about 0.1% concentration)

By far the most important application of solid scintillation crystals is in the detection and measurement of γ-rays. Scintillation detectors have three distinct advantages over G-M tubes for measurement of γ-rays. These are:

(1) Higher detection efficiencies (20 to 40 times);
(2) No significant resolving time corrections up to 10^5 counts/min;
(3) The output pulse-height is proportional to the γ-ray energy lost in the crystal. Therefore γ-ray spectrometry is possible.

NaI is hygroscopic and is encased in an air-tight metal can. The detectors are single crystals and should be protected against mechanical shock or temperature changes. The crystal and photomultiplier tube combination is generally sealed in an air-tight metal case and shielded by lead in the counting position. In spite of shielding, the background of a scintillation counter will generally be considerably higher than for a G-M counter. This is due partly to electronic noise but mostly due to the high efficiency of NaI(Tl) for background γ-rays. A lower-level pulse-height discriminator is used to reject noise pulses, since they are generally of smaller pulse-height than pulses due to the photons being measured.

The following four exercises are designed to illustrate the use and advantage of γ-ray scintillation counting.

2.1.1. Integral scintillation counting

MATERIALS AND EQUIPMENT

1. NaI(Tl) scintillation crystal – photomultiplier detector assembly.
2. Single-channel pulse-height analyser and scaler.
3. High-voltage supply.
4. Gamma-ray sources of ^{137}Cs, ^{60}Co and ^{133}Ba approximately 0.1 μCi each.

PROCEDURE

(1) Set the lower-level discriminator to 5 V. Set the differential-integral switch to integral or else disable the upper-level discriminator. The scaler will now count all pulses exceeding the lower-level setting. This is termed integral counting.

(2) Set the amplifier gain controls to their minimum value.

(3) Insert one of the above sources in the counting chamber.

(4) Increase the high voltage to approximately 600 V and turn the scaler to the count mode.

(5) Now increase the amplifier gain until the scaler begins counting.

(6) Keep the gain constant and begin recording count rate with increasing high voltage. Increase the high voltage in steps of 50 V. Do not increase the high voltage above 1500 V or serious damage to the photomultiplier tube will result.

(7) Repeat counting background only.

(8) Repeat with the other two sources.
Plot the above data as counts/min versus high voltage on 4 or 5 cycle semi-log graph paper.

(9) Using one of the three sources increase the gain of the amplifier by a factor of two and repeat the characteristic curve previously obtained. What shift do you note? What change in photomultiplier tube voltage is equivalent to a gain in two in amplified gain?

(10) Leave the gain the same as in (9) but increase the lower-level discriminator by a factor of two. You should obtain the original curve for your source. Why?

(11) If the activities of the three sources are known, plot counting yield (counts/γ-ray) versus energy in the highest counting flat portion of your curve. The geometry should be essentially the same for all sources. Explain the variation in counting yield versus source γ-ray energy. (Be sure and consider the number

of γ-rays per disintegration from the decay schemes as well as the half-life and age of the source.)

(12) Set the high voltage at the value corresponding to the start of the plateau for the ^{60}Co curve. Use the ^{60}Co source and with the lower level discriminator set at 2 V take counts with increasing discriminator setting until the count rate reaches background. Plot the integral curve. Explain the inflections of the slope of the curve.

(13) What settings would you use to measure ^{60}Co in the presence of ^{137}Cs?

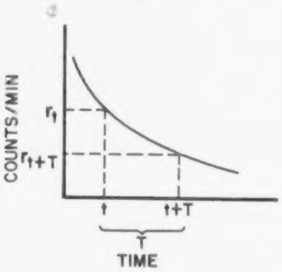

FIG. 24. Curve showing exponential decay of the activity of a sample where r_t is the count rate at the start of the counting interval, and r_{t+T} is the count rate at the end of interval T.

2.1.2. Rapid radioactive decay

INTRODUCTION

The primary purpose of this exercise is the investigation of the radioactive decay law in one short laboratory period. In this a short-lived radionuclide is observed, and a secondary effect (of less importance) may be considered. In counting of radionuclides with a very short half-life the length of the counting period must be considered as follows.

Consider the case of counting a radionuclide in which the counting period is long compared with the half-life of the nuclide, i.e. there is appreciable change in source activity over the counting interval.

Figure 24 shows the change in count rate over a counting period T.

The observed count rate then is equal to the counting yield times the total number of atoms that decayed during period T or

$$r_{obs} = Y\left[\frac{N_t - N_{t+T}}{T}\right] \qquad (1)$$

where N_t = number of atoms present at time t
N_{t+T} = number of atoms present after interval T

Since $N_{t+T} = N_t e^{-\lambda T}$
then

$$r_{obs} = Y \frac{N_t(1 - e^{-\lambda T})}{T} \tag{2}$$

If numerator and denominator are multiplied by λ then

$$r_{obs} = Y \frac{\lambda N_t(1 - e^{-\lambda T})}{\lambda T} \tag{3}$$

or

$$r_{obs} = \frac{r_t(1 - e^{-\lambda T})}{T} \tag{4}$$

Therefore, the count rate at the beginning of a counting period T may be related to the count rate observed over the whole period by

$$r_t = \frac{r_{obs}\, \lambda T}{(1 - e^{-\lambda T})} \tag{5}$$

Inspection of Eq. (5) reveals that if T is small compared with the half-life then λT will be small and for $\lambda T < 0.05$, $(1 - e^{-\lambda T})$ approaches λT.

With the aid of Eq. (5) the ratio r_t/r_{obs} may be calculated for various counting periods. If the duration of each counting period T is the same throughout a series of consecutive counts, then the ratio r_t/r_{obs} will be constant.

In the following experiment, Eq. (5) will be used to correct the count rate of ^{137m}Ba. ^{137m}Ba is the metastable isomer of ^{137}Ba. It has a half-life of 2.6 min and decays by emission of a 0.662-MeV gamma-ray.

MATERIALS AND EQUIPMENT

(1) SO_3-H type cation exchange resin (e.g. Amberlite IR-120 or Dowex-50).
(2) About 200 μCi ^{137}Cs in solution.
(3) A conventional burette.
(4) EDTA solution (approximately 0.3% adjusted to pH 11-12 with NaOH).
(5) Plastic counting container.
(6) Stop-watch.

PROCEDURE

(1) Saturate 50 g of resin with Na^+ by leaving it overnight in 10% NaCl solution. Put a glass-wool plug at the bottom of the burette, and fill half up with resin. Run 1 litre of distilled water upwards

through the resin to remove excess NaCl and air bubbles. Allow the resin to settle and wash with EDTA solution. (Never allow the surface of the EDTA solution to come below the top of the resin column.)

(2) Lower the surface of the EDTA solution to the top of the resin and apply the ^{137}Cs. Elute the column repeatedly with EDTA solution at 10-min intervals until the amount of ^{137m}Ba coming through each time no longer increases. At each elution the ^{137m}Ba concentration in the effluent will be maximum after about a half or a third of the resin-column volume of EDTA solution has run through (but the peak is not sharp).

(3) Take about 0.5 ml of the effluent rich in ^{137m}Ba in a plastic counting container; start counting immediately, using the scintillation detector (well-type if available).

(4) The counting should be carried out at 1-min intervals for a duration of 1 min counting time and a total running time of 30 min without removal of the sample container.

(5) At the end of 30 min from the starting time of counting, the ^{137m}Ba remaining in the liquid will be much less than 1% of the original, and most of the count rate observed above empty-container background results from some ^{137}Cs leached by the EDTA effluent.

(6) Repeat counting for 1 min at 5-min intervals until the count rate no longer decreases, and subtract the final count rate from the observed count rate and plot this net count rate from ^{137m}Ba against time on semi-log paper. Deduce the half-life of ^{137m}Ba from this plot.

(7) Use Eq.(5) to obtain the net count rates at the beginning of each counting period, and plot these corrected values of net count rate against time on semi-log paper.

2.1.3. Inverse-square law; attenuation of gamma rays

INTRODUCTION

The intensity of the γ-rays from a source of radiation can be reduced by (a) increase in distance between the source and the point of observation and (b) an increase in the shielding material in the path of the γ-rays.

(a) For a point source, the radiation intensity is conversely proportional to the square of the distance if no intervening matter is present in between. This is usually referred to as the "inverse-square law", and applies to all electromagnetic radiation. If the intensity of any source is known at any distance, then it can be calculated at any other distance by

$$\frac{I_1}{I_2} = \frac{d_2^2}{d_1^2} \qquad (1)$$

In this exercise, the decrease due to distance of γ-rays from a small source is investigated.

MATERIALS AND EQUIPMENT

(1) ^{60}Co or ^{137}Cs source approximately 5-10 μCi.
(2) NaI(Tl) scintillation counter system.

PROCEDURE

(1) Apply an operating voltage previously determined in Experiment 2.1.1 for the scintillation counter.
(2) Determine the background count rate.
(3) Determine the count rate as a function of distance from the source. Increase the distance until the background count is obtained.
(4) Plot the net count rate versus the distance on log-log graph paper and draw the best straight line through the points.
(5) Determine the slope and explain the reason for discrepancies, if any, with the inverse-square law.
(6) Calculate the exposure dose rate in mR/h at 1 metre from the source.

(b) The attenuation by matter of a collimated beam of monoenergetic γ-ray photons is exponential. (See Section 3.2.1 of the Lecture Matter.) The attenuation of γ-rays from ^{60}Co (or ^{137}Cs) will be investigated.

MATERIALS AND EQUIPMENT

(1) ^{60}Co or ^{137}Cs source approximately 5-10 μCi.
(2) NaI(Tl) scintillation counter system.
(3) Lead shielding.
(4) Absorber set, preferably lead.
(5) Micrometer for measuring absorber thickness.

PROCEDURE

(1) The previous experimental set-up is used except that the distance between the sample and counter is now fixed. The source should be collimated by lead shielding.
(2) Determine the count rate of the sample without adding any absorber between the source and detector.
(3) Determine the count rate after placing one absorber between source and detector. Repeat with increasing amounts of absorber.
(4) Remove the source completely and determine background with all the absorbers in place.
(5) The net count rate from the sample is proportional to γ-ray intensity and this is plotted against linear absorber thickness (mm) on semi-log paper.

(6) Determine the half-thickness of lead for ^{60}Co (or ^{137}Cs).
(7) Explain any discrepancy with simple exponential attenuation, i.e. any lack of straight line on semi-log paper.

2.2. Liquid scintillation counting

INTRODUCTION

Liquid scintillation counting has the advantage that the sample is dissolved in the liquid organic fluor and there is no self-absorption of the beta (or alpha) particle. For this reason, liquid scintillation counting is the method of choice for counting low-energy β-emitters. Both ^{3}H and ^{14}C, the two most important radiotracers in biological research, emit only β-particles of very low energy. Organic fluors also exhibit a very short decay time of the fluorescence produced and resolving-time corrections are not necessary. In addition, liquid scintillation counters can be adopted to sample changers which allow automatic counting of large numbers of samples.

The general description of a liquid scintillation counter is found in the Lecture Matter, Section 2.3.2. The following experiment is designed to illustrate its use including methods to correct for "quenching". An optional exercise demonstrating Čerenkov counting is also presented.

MATERIALS AND EQUIPMENT

(1) Liquid scintillation counter – refrigerated or ambient temperature (similar to Packard Instrument Co. Model 314 EX series).
(2) Liquid fluor system:

Toluene	0.5 litres
PPO (2-5 diphenyloxazole)	4 g/litre
POPOP (1,4-bis-2-5 phenyloxazolyl)	0.05 g/litre

PPO is the primary solute. POPOP is termed a secondary solute and is used to shift the fluorescence spectrum of PPO to longer wavelengths for better matching to the spectral response of photomultiplier tubes. This procedure increases the counting yield and is generally used in measurement of ^{3}H.

(3) Benzoic acid-7-^{14}C dissolved in toluene – approx. 0.02 μCi/ml. Benzoic acid-^{3}H dissolved in toluene – approx. 0.1 μCi/ml (or other available compounds of ^{14}C and ^{3}H soluble in toluene).
(4) Chloroform.
(5) Sample counting vials.

2.2.1. Determination of optimal counter settings

PROCEDURE

(1) Take sufficient time to read the instrument instruction manual and become thoroughly familiar with operating controls.

(2) Fill the sample counting vials with an equal measured volume of the liquid scintillation solution (15 ml). (Pipette accuracy is not necessary.)

(3) Pipette 100 λ of the ^{14}C solution into one vial.
Pipette 100 λ of the ^{3}H solution into one vial.

Use one vial containing only the liquid scintillation solution as a background vial.

(4) For a dual-channel instrument:

Set the lower discriminator of channel A at 10 V.
Set the upper discriminator of channel A at 50 V.
Set the lower discriminator of channel B at 50 V.
Set the upper discriminator of channel B at 100 V (or ∞).

Channel A will now record all pulses with pulse heights between 10 V and 50 V and channel B will record all pulses with pulse heights between 50 V and 100 V (or ∞).

(5) Determine the background count rate r_b in both channels as a function of increasing high voltage. Begin at about 600 V.

(6) Determine the count rate of each sample in both channels as a function of increasing high voltage.
At one particular voltage, the count rate in channel A will be approximately equal to the count rate in channel B. This voltage will very closely correspond to what is known as balance point operation. At this point, a decrease in counts from channel A due to quenching will be offset by additional counts from B due to quenching. Balance point operation provides a condition of high counting yield and at the same time a minimum sensitivity to quenching. The balance point settings will not be the same for the two radionuclides.

(7) Determine the apparent counting yield (Y) at the balance point settings for each radionuclide. It is not known at this point the extent of quenching (if any) in the benzoic acid standards.

2.2.2. Determination of counting yield at the optimal settings

This will be accomplished by the method of "Internal Standardization" or spiking. After counting at the optimum settings an aliquot of a standard (known disintegration rate) is added to each sample. Toluene-^{14}C and toluene-^{3}H (which are unquenching standards) may be used and must be added very accurately. An amount approximately equal to the sample count rate should be added. (The pipetting of labelled toluene is somewhat difficult and practice with unlabelled toluene should be taken.)

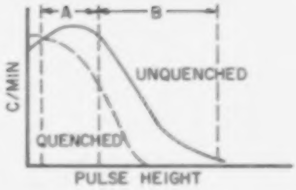

FIG. 25. Effect of quenching on beta spectrum in liquid scintillation counter.

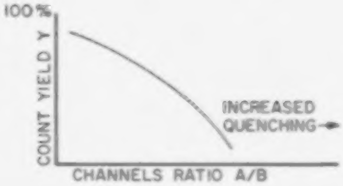

FIG. 26. Quench correction curve.

TABLE VI. QUENCH CORRECTIONS

Sample No.	Liquid fluor system PPO (4 g/l) and POPOP (0,05 g/l) (ml)	Toluene (ml)	Chloroform (ml)
1	14	1	0
2	14	0,9	0,1
3	14	0,8	0,2
4	14	0,5	0,5
5	14	0,25	0,75
6	14	0	1,0

After spiking, recount the samples and calculate the counting yield Y by the following

$$Y = \frac{r_{\text{standard + sample}} - r_{\text{sample}}}{\text{disintegration rate of standard}} \tag{1}$$

Are the counting yields calculated above the same as determined previously for the benzoic acid samples? Does this indicate any quenching effect by the benzoic acid?

2.2.3. Determination of counting yield by the channels ratio method

This is a very simple method for quench correction and can be accomplished using any counter with two or more channels.

As mentioned previously, the effect of quenching is to decrease the energy (increase the wavelength) of the fluorescence spectrum produced by the β-emitter. This is illustrated in Fig. 25.

As can be seen from Fig. 25, the ratio of counts in channels A/B increased as the quenching occurred. If increasing amounts of quenching agent are added to a series of samples of known activity, then a correction curve as shown in Fig. 26 may be constructed.

This procedure has been shown to be generally independent of the nature of the quenching agent.

PROCEDURE

(1) Prepare a series of counting samples with compositions as prescribed in Table VI. Chloroform will serve as the quenching agent.

(2) To each of the samples add 100 λ of a standard solution of toluene-^{14}C containing 0.01 μCi/ml (or similar standard solution).

(3) At the optimum settings for ^{14}C, count each sample in the series. Determine the net count-rate ratio of channel A/B. Plot the A/B ratio versus counting yield as in Fig. 26.

2.2.4. Čerenkov counting in a liquid scintillation counter

The purpose of this exercise is to demonstrate the use of a liquid scintillation counter in detection of high-energy β-emitters. Water serves as the dielectric medium for production of Čerenkov light and scintillation fluid is not necessary.

MATERIALS AND EQUIPMENT

(1) Standard radioactive solutions of ^{42}K or ^{32}P approximately 0.01 μCi/ml in aqueous solution.

PROCEDURE

(1) Fill one counting vial with water and one with standard liquid fluor solution.

(2) Add 100 λ of the ^{42}K (or ^{32}P) solution to each.

(3) Determine the counting yields for Čerenkov counting and liquid scintillator counting.

The efficiency of Čerenkov light production increases rapidly with the energy of the beta particles' energy. The threshold for the effect is approximately 0.3 MeV.

Discuss the possible advantages of Čerenkov counting in biological research.

3. DECONTAMINATION

INTRODUCTION

In work with radioactive materials, it is always necessary to know if the operator or the procedure is causing contamination of personnel, the equipment or the laboratory.

Often a fresh spill on a clean and polished surface can be washed or absorbed without resultant detectable contamination. If it is allowed to react with the surface, however, drastic action might be required to remove it.

The methods of decontamination may be divided into physical and chemical. Physical methods include absorption, vacuum cleaning, and polishing, and steam or sand blasting. Chemical methods include the use of acids and alkali with or without carrier, detergents, complexing agents and ion-exchange material.

This important experiment will consist in contamination of several different materials with several radionuclides and subsequent decontamination with commonly used chemical agents.

MATERIALS AND EQUIPMENT

(1) Solutions of ^{32}P, ^{45}Ca and ^{131}I, approximately 1 μCi/ml.
(2) Small pieces of the following materials: glass, lead, waxed linoleum, perspex or lucite, stainless steel, painted wood and brick or masonry stone.
(3) Wash solution: a 1% detergent solution of 0.3% EDTA to which is added NaOH to bring the pH to 12. "Radiacwash" is a commercial preparation of this type.
(4) 1% carrier solutions of P, Ca and I, e.g. Na_3PO_4, $Ca(NO_3)_2$ and NaI.
(5) 2$\underline{N}$ HCl, acetone.
(6) Absorbent tissue paper.
(7) G-M counter-scaler system.

PROCEDURE

(1) Count each material to be tested in the shielded G-M counter system.
(2) Using normal radiochemistry laboratory precautions, e.g. protective gloves, apron, etc. (see Section 3.2, Lecture Matter) contaminate each material by pipetting 100 λ of each radioactive solution on the surface and drying.
(3) Count each material in the G-M counter and record as initial activity.
(4) Wipe each surface with a damp tissue paper, dry and measure residual activity.
(5) For a second series, use "washing solution" as decontaminating material. Dry the sample, and measure the activity.
(6) Try all the relevant decontaminating agents in a similar manner. If necessary, as a final step with linoleum, try removing the wax with acetone.
(7) Record all the measured activities, and compare the effect of decontaminating agents upon various substances and radioactive isotopes.

(8) Determine the extent of contamination of the protective gloves worn during the experiment.

4. EXERCISES ON BASIC APPLICATION

4.1. Combustion of carbon compounds (determination of specific activity by persulphate oxidation of compound)

INTRODUCTION

It is not always possible to compare specific activities of diverse labelled carbon compounds because of differences in crystal structure, ease of crystallization etc. Consequently, it is necessary to convert all such compounds to a common one. A convenient one is carbon dioxide, which, for practical purposes, we measure as the barium salt, $BaCO_3$.

Combustion may be performed in two major ways: "dry" combustion and "wet" combustion. In dry combustion the sample is burned in the usual manner with CuO for determination of formula composition, except that the CO_2 is caught quantitatively in an insoluble form, that is, either as the gas or as carbonate. In wet combustion the compound is dissolved in a solution which includes the oxidants. Two commonly used solutions, the Van Slyke combustion mixture (H_2SO_4, Cr_2O_7, KIO_3, H_3PO_4) and the persulphate mixture, differ sharply in their response according to the amount of water present. The utility of the former diminishes rapidly with increasing water content whereas the latter uses water as a solvent. The convenience of water as a solvent may well be an over-riding factor, as is illustrated in this experiment.

Oxidation of an aqueous solution of a variety of organic compounds (including acetate, succinate, glucose and acetone) may be accomplished quantitatively with potassium persulphate ($K_2S_2O_8$) at about 100° C. The CO_2 evolved is quantitative and is caught in a NaOH gas-washing bottle and converted to $BaCO_3$ for plating.

REAGENTS AND MATERIALS

(1) $CH_3{}^{14}COONa$.
(2) 200 mg $K_2S_2O_8$.
(3) 1 ml 5% $AgNO_3$.
(4) 0.1N NaOH, CO_2-free.
(5) H_2O, CO_2-free.
(6) $BaCl_2$ (saturated aqueous solution).
(7) CH_3OH, abs.
(8) N_2 gas, or CO_2-free air.
(9) Flow apparatus, consisting of a two-necked reaction flask, an H_2O condenser and a gas-washing tube.
(10) Filter apparatus (chimney and filter-stick).
(11) Centrifuge.
(12) Infra-red lamp.
(13) Analytical balance.
(14) Calibrated standard (i.e. infinitely thin ^{14}C source of usual sample area and containing known number of dis/min).

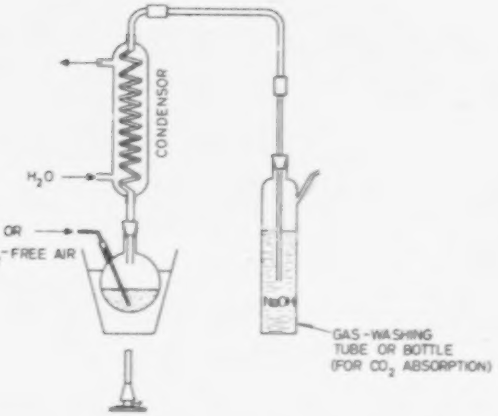

FIG. 27. Assembly for combustion of carbon compounds and conversion of CO_2 produced.

PROCEDURE

(1) Arrange the flow apparatus as shown in Fig. 27.
(2) Calculate the amount of $CH_3{}^{14}COONa$ needed to produce approximately 50 mg of $BaCO_3$, and place into the reaction chamber.
(3) Add about 20 ml H_2O, 200 mg $K_2S_2O_8$ and 1 ml 5% $AgNO_3$. (No reaction occurs at room temperature.)
(4) Heat the reaction vessel to 70° C for 20 min; then increase the temperature slowly to boiling, and maintain until the solution becomes clear or the persulphate is dissolved.
(5) Simmer 10 min longer and sweep the system for an additional 10 min to remove all traces of $^{14}CO_2$.
(6) Wash the inlet of the gas-washing system with CO_2-free water combining the washings with the 0.1N NaOH.
(7) Add sufficient saturated $BaCl_2$ solution to precipitate all the $^{14}CO_2$.
(8) Transfer quickly to a centrifuge tube, and centrifuge immediately (or stopper and centrifuge at will).
(9) Wash the precipitate once with CO_2-free water and then with abs. CH_3OH.
(10) Re-suspend in CH_3OH, and filter onto the filter apparatus.
(11) Place an infra-red lamp 4-5 cm above the plating apparatus, and continue drawing warmed air through the apparatus for 10-15 min, by which time the $BaCO_3$ plate will have dried.
(12) Weigh immediately.
(13) Count sample and standard.
(14) Calculate the absolute specific activity of sample (dis/min per mmole), making corrections for self-absorption and taking the counting yield of the standard into account.

QUESTIONS

1. (1) State the conditions for preference for persulphate versus Van Slyke oxidation procedures.
2. (2) What is the fate of the persulphate in the reaction?

4.2. Tracer dilution chemistry

INTRODUCTION

One of the important advantages of using a radioactive substance in quantitative analysis is that a quantitative isolation of the compound to be determined from a material is unnecessary. A simple isotopic dilution analysis of the phosphorus concentration in an unknown solution by comparison with a solution of known phosphorus concentration will be conducted in this experiment. The radioisotope technique illustrated by this experiment is advantageous in any situation where a normal quantitative determination of the test substance is not feasible for some reason.

REAGENTS AND MATERIALS

(1) Solution containing 0.20 mmole P/ml solution.
(2) Unknown P solution (of the order of 0.1$\underline{M}$).
(3) Solution containing about 0.1 μCi ^{32}P ("carrier-free" or of known P concentration).
(4) Fiske's reagent (13 g MgO, 175 g citric acid, 330 ml 25% NH_4OH in water to give a 1-1 solution.
(5) 25% NH_4OH.

PROCEDURE

(1) Mark six 100-ml beakers as U_1, U_1', U_2, U_2', K and K', and pipette into them, respectively, the following aliquots:

U_1 U_1'	5 ml unknown
U_2 U_2'	20 ml unknown
K K'	5 ml of 0.20$\underline{M}$ H_3PO_4 solution

(2) Pipette accurately 1 ml of active phosphate solution into each beaker, and mix thoroughly.
(3) Add slowly 10 ml of Fiske's reagent and 10 ml of 25% NH_4OH while swirling.
(4) After 5 min decant the supernatant from the precipitates; wash three times with distilled water and once with methanol.
(5) Transfer the major part of the precipitates into weighed and marked counting cups. The amount of thick slurry of the precipitate transferred from the K-beakers should be roughly in between the amount from the U_1- and U_2-beakers, respectively.
(6) Dry the thick slurry under an infra-red lamp, trying to make the surface even.
(7) After cooling, weigh the cups plus precipitates, and determine weights of precipitate alone.

(8) Count the activity, using a G-M counter.
(9) Express the specific activities of P in the solid samples in cpm per mmole.
(10) Calculate the molarity of the unknown P-solution.

QUESTIONS

(1) Do the values obtained for U_1 and U_2 come out the same?
(2) What difference does it make to the calculation of unknown P conc. if the activities of the samples are expressed as cpm per mg precipitate? Explain.
(3) Can the unknown P conc. be determined from the weights of the precipitates alone?

4.3. Kinetics of exchange between ions in solution and those in solid form

INTRODUCTION

It is often observed that at equilibrium the total concentration of a substance distributed in two phases remains constant with respect to time. This, however, does not imply that the individual ionic or molecular species is restricted in one phase. Instead, dynamic exchange of ionic or molecular species between the two phases is continually taking place.

By the introduction of a radioactive tracer, it is possible to investigate the dynamic exchange of a species under equilibrium conditions.

In this experiment, the exchange of ions between a solution and a solid surface will be studied by observation of the approach to tracer equilibrium.

The necessary equations for this system were developed in Lecture Matter, Section 5.3, Equations (44-53). The system can be analysed as a closed, two-compartment model in the steady state.

The model in Fig. 28 describes the system:

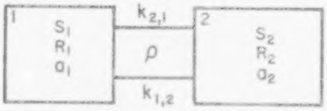

FIG. 28. Model of a two-compartment system in the steady state.

In Fig. 28 let compartment 1 be the solution and compartment 2 represent the solid surface.

S_1 = stable isotope (tracee) in solution
S_2 = stable isotope (tracee) in the solid
R_1 = activity (cpm) of tracer isotope in solution
R_2 = activity (cpm) of tracer isotope in solid
a_1 = specific activity cpm/mmole in solution
a_2 = specific activity cpm/mmole in solid
at t = 0 the label is added to the solution, therefore $a_1(0) = R/S_1$
where R = total tracer activity

If now the system is sampled as it approaches tracer equilibrium, the first-order rate constants $k_{1,2}$ and $k_{2,1}$ and the exchange rate may be determined as developed in Eqs (44-53).

MATERIALS AND EQUIPMENT

(1) Anion-exchange resin, $H_2PO_4^-$ saturated in solution (3).
(2) 5 μCi ^{32}P (high specific activity).
(3) Solution of 10^{-3} $\underline{M}$ KH_2PO_4.
(4) Pipettes and fine-mesh cloth pipette tip caps (pro-pipettes).
(5) Stop-watch.
(6) G-M counter.

PROCEDURE

(1) Measure 2 ml of wet cation-exchange resin into a 150-ml beaker.
(2) Add 100 ml of KH_2PO_4 solution.
(3) Now add the 5 μCi ^{32}P in a 10-ml aliquot of the phosphate solution, begin stirring and simultaneously start the stop-watch. Take out 1 ml of the supernatant solution by pipette into a planchet at the following times: 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40 and 60 min after addition of tracer.
It is important to avoid getting resin particles into the pipette. The tip of the pipette must be covered with a fine-mesh cloth cap during filling. The cap is removed during emptying, and replaced before the next filling. Between sampling the solution should be continuously stirred to eliminate the activity concentration gradient near the resin particles.
(4) Dry the samples (under the infra-red lamp) and determine the activity in each sample using the G-M counter.
(5) Since the concentration of P in solution was constant, the count rate per ml of solution is proportional to a_1. $a_1(0)$ may be determined by extrapolation or by calculation. If $a_1 - a$ (the equilibrium value) is plotted versus time on semi-log graph paper, then the slope may be determined and the exchange rate of P between solid and solution as well as the two rate constants. In this instance, S_1 is known and S must be determined from the equilibrium value and the S_2 by difference.
Calculate the exchange rate in mmole/min. There is no chemical procedure to determine this value.

4.4. Neutron activation analysis for silver

This exercise will serve to illustrate the neutron activation analysis technique.

Natural silver consists of isotopes of mass 107 and mass 109 in about equal abundance. Upon neutron capture, ^{107}Ag yields ^{108}Ag, which has a half-life of 2.42 min, and ^{109}Ag yields ^{110}Ag, which has a half-life of 0.41 min. Both radionuclides are energetic β-emitters and may be detected easily by G-M counting. In this experiment, the amount of silver in a coin will be estimated by the comparator method. Because of the very short half-lives of the silver isotopes, the unknown (coin) and the comparator will be separately irradiated but in precisely the same way.

MATERIALS AND EQUIPMENT

(1) Moderated Laboratory Neutron Source – a 5-Ci Pu-Be source or similar.
(2) Coin of appreciable silver content.
(3) Pure silver foil.
(4) G-M counter.

PROCEDURE

(1) Weigh the coin and record the weight.
(2) Weigh the silver foil comparator (cut to the same shape and size as the coin) and record the weight.
(3) The remaining procedure should be duplicated for the coin and the comparator.
(4) Irradiate for exactly 10.0 min by the neutron source. Ensure there is approximately 6-8 cm of paraffin between the source and sample position.
(5) Terminate the irradiation and quickly transfer the sample to the G-M counter.
(6) Exactly 30 sec after the irradiation start counting for an interval of 24 sec. Record the scaler reading, quickly reset and start a second 24-sec count after the irradiation.
(7) Continue taking two 24-sec counts per minute until 7 min after the irradiation.
(8) Subtract the background from each count and plot the log of the net count rate versus corresponding time after irradiation.
(9) Since the net count rate will be due to the activity of both silver isotopes with two different half-lives then the above plot will be a sum of two separate exponential functions. These can be analysed by extrapolating the straight portion of the observed count rate to zero time. This line should have a half-life of 2.42 min. If this line is subtracted from the observed count rate a second exponential will result. The half-life of this second exponential should be 24 sec.
(10) Determine the ratio of the intercepts of the two exponential functions. This should be the ratio of the activities of the two silver isotopes ^{108}Ag/^{110}Ag. This ratio should be approximately the ratio of the activation cross sections of ^{107}Ag/^{109}Ag. Compare with published data.
(11) Determine the silver content of the coin assuming the comparator is 100% silver.
(12) What assumptions were used in the above calculations?

4.5. Determination of copper in biological material by neutron activation analysis

INTRODUCTION

Because of the low Cu content of biological materials, any chemical means to determine it quantitatively requires large amounts of material.

Activating the Cu present in biological material with neutrons has the advantage of requiring only a small sample for a quantitative determination.

Copper-63, which is 69.2% abundant in natural copper, is activated to ^{64}Cu, which has a half-life of 12.75 h and emits β-particles of 0.57 MeV and γ-rays of 1.34 MeV. Activation for one $T_{\frac{1}{2}}$ (12.8 h) gives a specific activity of $\cong$ 50 mCi/g. Simultaneously, of course, ^{65}Cu is activated to ^{66}Cu, but its half-life of 5.1 min is too short for the chemical separation procedures that are necessary.

In this experiment the Cu content of plant or animal tissue is determined by the comparator method. After activation, the tissue is digested, Cu carrier is added and mixed with the radioactive Cu. Cu is then separated non-quantitatively by a chemical method and weighed and the ^{64}Cu is counted. The specific activity of the unknown sample is then compared with the specific activity of a known Cu standard and the Cu content of the original tissue sample is calculated.

REAGENTS AND EQUIPMENT

(1) $24\underline{N}$ HNO_3 (conc.)
$16\underline{N}$ HNO_3 (conc.)
$\underline{N}$ HNO_3
$18\underline{N}$ CH_3COOH (conc.)
$15\underline{N}$ NH_4OH (conc.)
$2\underline{N}$ NH_4OH
Acetone
SO_2-saturated water
Cu carrier (20 mg Cu/ml): 6.28 g $Cu(CH_3CO_2)_2H_2O$ in 100 ml water
15% Na_2SO_3 (wt/vol.)
20% KSCN (wt/vol.)
10% $Fe(NO_3)_3$ (wt/vol.)
10% $NH_4H_2PO_4$ (wt/vol.)
2% salicylaldoxime in ethanol

(2) Reactor or neutron generator capable of 10^{12} n/cm^2 sec fluxes.

PROCEDURE

Take about 0.05 g of tissue and 1 μg of Cu standard sealed in polythene. Activate for 13 h in identical neutron fluxes.

(1) In a hood, transfer tissue and standard to 50-ml centrifuge tubes, and add 10 drops of $24\underline{N}$ HNO_3. Boil until tissue has dissolved, and add 10 mg of Cu (0.5 ml of Cu carrier). Make up to 4 ml with water; add 1 ml of Na_2SO_3 and 1 ml of KSCN. Boil, and spin down CuSCN when it has settled, reject supernatant and wash precipitate with hot water saturated with SO_2.

(2) Dissolve precipitate in 0.5 ml of hot $16\underline{N}$ HNO_3; add 5 drops of $Fe(NO_3)_3$ and 1 drop of $NH_4H_2PO_4$, then $15\underline{N}$ NH_3 till dark brown. Boil, spin down $Fe(OH)_3$ precipitate and wash it once with $2\underline{N}$ NH_3.

(3) Combine supernatant and washings in a fresh tube, and acidify with CH_3CO_2H till pale blue. Then add 0.5 ml of $16\underline{N}$ HNO_3, 1 ml of Na_2SO_3 and 1 ml of KSCN; boil and spin down CuSCN. Pour away supernatant and wash precipitate with hot water saturated with SO_2.

(4) Dissolve precipitate in 0.5 ml of $16\underline{N}$ HNO_3; add $15\underline{N}$ NH_3 until solution is deep blue and CH_3CO_2H until it is pale blue. Add 3 ml of salicylaldoxime, and boil for 3 min. Spin down precipitate, and wash it twice with water and once with acetone.

(5) Slurry precipitate with acetone onto a weighed aluminium counting tray; dry under a lamp, and count with a NaI(Tl) scintillation counter. Correct counts for decay and check the half-life of the separated ^{64}Cu. The chemical steps take about 2 h for eight samples. The chemical yield is about 75%.

CALCULATION

$$\mu g \text{ Cu in sample} = \frac{\text{cpm sample}}{\text{cpm standard}} \times \frac{\text{wt standard Cu-salicylaldoxime}}{\text{wt sample Cu-salicylaldoxime}}$$

RANGE AND ACCURACY

0.05-0.5 μg of Cu is a convenient range for determination within an accuracy of 5%.

INTERFERENCES

Large amounts of zinc might interfere because of the reaction $^{64}Zn(n, p)^{64}Cu$. 1 μg of zinc in the sample yields ^{64}Cu equivalent to 7×10^{-4} μg of copper.

MENTAL EXERCISES

(1) When the Z-number of all nuclides is plotted against the N-number, isotopes of a particular element will be found on a horizontal line. This kind of representation is usually given on nuclear charts. How can the decay products of a particular nuclide be found after the emission of

α-particle?
β^--particle (electron)?
β^+-particle (positron)?
γ-ray?
  electron capture (K-capture)?
  internal conversion?
  neutron?

(2) With the aid of a nuclear chart, find the decay products of ^{14}C, ^{22}Na, ^{40}K, ^{90}Sr and ^{238}U.

(3) Calculate the weight of 100 μCi of carrier-free ^{14}C and 100 μCi of carrier-free ^{22}Na.

(4) If a solution has a concentration of 100 μCi of carrier-free ^{14}C per ml, calculate its molarity.

(5) A sample of ^{60}Co has an activity of 1 Ci, calculate its activity 2 years later.

(6) A radionuclide has lost 15/16 of its original activity in 32 min. Calculate the half-life of the nuclide.

(7) ^{137}Ba is formed from ^{137}Cs. How many mCi of ^{137m}Ba will be formed from 100 mCi of ^{137}Cs in 1, 2 and 20 min?
Answer: 23.9 mCi, 42.0 mCi and 99.57 mCi.

(8) Determine the daily decrement (in per cent) of activity of any ^{32}P preparation.

(9) A ^{24}Na sample ($T_{\frac{1}{2}}$ = 14.8 h) had a counting rate of 24 000 cpm. One hundred hours later it gave 250 cpm. Roughly estimate the dead-time of the G-M counter.

(10) The activity of ^{14}C in 8 g of natural carbon sample with background was found to have 10.2 cpm. The background of the counter was 4.5 cpm and the counting yield was 5%. Neglecting the statistical deviation calculate the ^{14}C content. $T_{\frac{1}{2}}$ = 5600 yr.

(11) The background counting rate of a G-M counter system is 30 cpm. A sample is counted giving a total of 4050 counts in 15 min. The background is counted for 5 min. Calculate the net count rate of the sample and the standard deviation as a percentage of the net count rate.

(12) A 0.1-mg sample of pure ^{239}Pu underwent 1.4×10^8 dis/min. Calculate the half-life of this radioisotope.

(13) Calculate the thickness of lead shielding necessary to reduce the exposure dose in air to 2.5 mR/h at 1 m from a 100-mCi ^{60}Co source. Hint: Use Eq. (22) and assume the half-thickness of lead for ^{60}Co γ-rays to be 1.3 cm. Answer: 7.5 cm.

(14) Indicate the increase or decrease in number of neutrons and protons and the mass number after the following nuclear reactions:

$$(n, p),$$
$$(n, \gamma),$$
$$(n, \alpha).$$

(15) Scandium is to be determined by the activation method. Assuming the lower limit of the determination to be 50 cpm at 10% G-M counting yield, compute the minimum amount of scandium determinable if the sample is subjected to a neutron flux of 10^{12} n/cm^2 sec for 2 h. Assume the (n, γ) reaction is the most likely reaction and that the shielding effect is negligible.

(16) What would be the specific activity of phosphorus having a cross-section of $\sigma = 0.2$ b after irradiation by a neutron flux of 10^{12} n/cm^2 sec for 1 h, 1 d and 10 d?

(17) Calculate the energy absorbed by a 70-kg man who has received a whole-body dose of 700 rads, an amount almost certain to be fatal. Assume the body to be of the specific heat of water and calculate the resulting temperature rise.
Answer: 117 calories, 1.7×10^{-3} degC.

APPENDIXES

APPENDIX I

INTEGRATION OF EQUATION (1). THE RADIOACTIVE DECAY LAW

Given

$$\frac{dN}{dt} = -\lambda N \qquad (1)$$

Separating variables for the indefinite integration

$$\int \frac{dN}{N} = -\lambda \int dt \qquad (2)$$

Now integrating

$$\ln N = -\lambda t + C \qquad (3)$$

where C is the constant of integration. C is evaluated at any initial conditions which are

$$N = N_0 \text{ at } t = 0$$

That is N_0 is the number of atoms present at any initial time.

Therefore, at

$$t = 0, \quad C = \ln N_0 \qquad (4)$$

Substituting (4) into (3)

$$\ln N - \ln N_0 = -\lambda t \qquad (5)$$

or

$$\ln\left(\frac{N}{N_0}\right) = -\lambda t$$

Now taking the antilogarithm

$$\frac{N}{N_0} = e^{-\lambda t} \qquad (6)$$

or

$$N = N_0 e^{-\lambda t} \qquad (7)$$

APPENDIX II

DERIVATION OF EQUATION (20) SECTION 2.5 IN LECTURE MATTER

From Eq. (19)

$$\sigma_{t_s} = \left(\frac{r_{s+b}}{t_s} + \frac{r_b}{t_b} \right)^{\frac{1}{2}} \qquad (1)$$

By taking the derivative of both sides

$$2\sigma_{t_s} \, d\sigma_{t_s} = - \frac{r_{s+b}}{t_s^2} \, dt_s - \frac{r_b}{t_b^2} \, dt_b \qquad (2)$$

To obtain the minimum value set $d\sigma_{t_s} = 0$; and since the allowed counting time is constant

$$t = t_s + t_b \qquad (3)$$

and

$$dt = dt_s + dt_b = 0 \qquad (4)$$

$$dt_s = - dt_b \qquad (5)$$

Therefore, rearranging Eq. (2)

$$\frac{t_b^2}{t_s^2} = - \frac{r_b}{t_s} \frac{dt_b}{dt_s} \qquad (6)$$

Therefore

$$\frac{t_b}{t_s} = \left(\frac{r_b}{r_{s+b}} \right)^{\frac{1}{2}} \qquad (7)$$

APPENDIX III

ABRIDGED VERSION OF TABLE OF RECOMMENDED MAXIMUM PERMISSIBLE CONCENTRATIONS OF RADIONUCLIDES IN AIR AND IN DRINKING WATER AND MAXIMUM PERMISSIBLE BODY BURDENS FOR OCCUPATIONAL EXPOSURE

Published in Report of Committee 2 ICRP (1962)

Radionuclide	Critical organ[a]	Maximum permissible concentrations				Maximum permissible burden in total body (μCi)
		For 40-h week		For 168-h week		
		$(MPC)_w$ μCi/cm³	$(MPC)_a$ μCi/cm³	$(MPC)_w$ μCi/cm³	$(MPC)_a$ μCi/cm³	
${}^{3}_{1}H$ (HTO or T_2O)[b] (sol.)	Body tissue	0.1	5×10^{-6}	0.03	2×10^{-6}	10^{3} 2×10^{3}
(T_2) (submersion)	Skin		2×10^{-3}		4×10^{-4}	-
${}^{7}_{4}Be$ (sol.)	GI (LLI) Total body	0.05	6×10^{-6}	0.02	2×10^{-6}	- 600
(insol.)	Lung GI (LLI)	0.05	10^{-6}	0.02	4×10^{-7}	- -
${}^{14}_{6}C$ (CO_2)[b] (sol.)	Fat	0.02	4×10^{-6}	8×10^{-3}	10^{-6}	300
(submersion)	Total body		5×10^{-5}		10^{-5}	-
${}^{18}_{9}F$ (sol.)	GI (SI)	0.02	5×10^{-6}	8×10^{-3}	2×10^{-6}	-
(insol.)	GI (ULI)	0.01	3×10^{-6}	5×10^{-3}	9×10^{-7}	-
${}^{22}_{11}Na$ (sol.)	Total body	10^{-3}	2×10^{-7}	4×10^{-4}	6×10^{-8}	10
(insol.)	Lung GI (LLI)	9×10^{-4}	9×10^{-9}	3×10^{-4}	3×10^{-9}	- -
${}^{24}_{11}Na$ (sol.)	GI (SI)	6×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
(insol.)	GI (LLI)	8×10^{-4}	10^{-7}	3×10^{-4}	5×10^{-8}	-
${}^{31}_{14}Si$ (sol.)	GI (S)	0.03	6×10^{-6}	9×10^{-3}	2×10^{-6}	-
	GI (ULI)	6×10^{-3}	10^{-6}	2×10^{-3}	3×10^{-7}	-

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[a] The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

[b] See word of caution, Section 3.2.2, Basic Part.

Radionuclide	Critical organ[a]	Maximum permissible concentrations For 40 h-week		For 168-h week		Maximum permissible burden in total body (μCi)
		$(MPC)_w$ (μCi/cm^3)	$(MPC)_a$ (μCi/cm^3)	$(MPC)_w$ (μCi/cm^3)	$(MPC)_a$ (μCi/cm^3)	
${}^{32}_{15}$P (sol.)	Bone	5×10^{-4}	7×10^{-8}	2×10^{-4}	2×10^{-8}	6
	Lung		8×10^{-8}		3×10^{-8}	-
(insol.)	GI (LLI)	7×10^{-4}		2×10^{-4}		-
${}^{35}_{16}$S (sol.)	Testis	2×10^{-3}	3×10^{-7}	6×10^{-4}	9×10^{-8}	90
(insol.)	Lung		3×10^{-7}		9×10^{-8}	-
	GI (LLI)	8×10^{-3}		3×10^{-3}		
${}^{36}_{17}$Cl (sol.)	Total body	2×10^{-3}	4×10^{-7}	8×10^{-4}	10^{-7}	80
(insol.)	Lung		2×10^{-8}		8×10^{-9}	-
	GI (LLI)	2×10^{-3}		6×10^{-4}		-
${}^{38}_{17}$Cl (sol.)	GI (S)	0.01	3×10^{-6}	4×10^{-3}	9×10^{-7}	-
(insol.)	GI (S)	0.01	2×10^{-6}	4×10^{-3}	7×10^{-7}	-
${}^{41}_{18}$Ar	Total body		2×10^{-6}		4×10^{-7}	-
(submersion)						-
${}^{42}_{19}$K (sol.)	GI (S)	9×10^{-3}	2×10^{-6}	3×10^{-3}	7×10^{-7}	-
(insol.)	GI (LLI)	6×10^{-4}	10^{-7}	2×10^{-4}	4×10^{-8}	-
${}^{45}_{20}$Ca (sol.)	Bone	3×10^{-4}	3×10^{-8}	9×10^{-5}	10^{-8}	30
(insol.)	Lung		10^{-7}		4×10^{-8}	-
	GI (LLI)			2×10^{-4}		-
${}^{47}_{20}$Ca (sol.)	Bone	10^{-3}	2×10^{-7}	5×10^{-4}	6×10^{-8}	5
(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	3×10^{-4}	6×10^{-8}	-
	Lung		2×10^{-7}		6×10^{-8}	-
${}^{46}_{21}$Sc (sol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	8×10^{-8}	-
	Liver		2×10^{-7}		8×10^{-8}	10
(insol.)	Lung		2×10^{-8}		8×10^{-9}	-
	GI (LLI)	10^{-3}		4×10^{-4}		-
${}^{47}_{21}$Sc (sol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	9×10^{-4}	2×10^{-7}	-
(insol.)	GI (LLI)	3×10^{-3}	5×10^{-7}	9×10^{-4}	2×10^{-7}	-

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[a] The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

Radionuclide		Critical organ[a]	Maximum permissible concentrations For 40 h-week		For 168-h week		Maximum permissible burden in total body (μCi)
			$(MPC)_w$ (μCi/cm^3)	$(MPC)_a$ (μCi/cm^3)	$(MPC)_w$ (μCi/cm^3)	$(MPC)_a$ (μCi/cm^3)	
$^{48}_{21}$Sc	(sol.)	GI (LLI)	8×10^{-4}	2×10^{-7}	3×10^{-4}	6×10^{-8}	-
	(insol.)	GI (LLI)	8×10^{-4}	10^{-7}	3×10^{-4}	5×10^{-8}	-
$^{48}_{23}$V	(sol.)	GI (LLI)	9×10^{-4}	2×10^{-4}	3×10^{-4}	6×10^{-8}	-
	(insol.)	Lung		6×10^{-8}		2×10^{-8}	-
		GI (LLI)	8×10^{-4}		3×10^{-4}		-
$^{51}_{24}$Cr	(sol.)	GI (LLI)	0.05	10^{-5}	0.02	4×10^{-6}	-
		Total body		10^{-5}		4×10^{-6}	800
	(insol.)	Lung		2×10^{-6}		8×10^{-7}	-
		GI (LLI)	0.05		0.02		-
$^{52}_{25}$Mn	(sol.)	GI (LLI)	10^{-3}	2×10^{-7}	3×10^{-4}	7×10^{-8}	-
	(insol.)	Lung		10^{-7}		5×10^{-8}	-
		GI (LLI)	9×10^{-4}		3×10^{-4}	5×10^{-8}	-
$^{54}_{25}$Mn	(sol.)	GI (LLI)	4×10^{-3}		10^{-3}		-
		Liver		4×10^{-7}		10^{-7}	20
	(insol.)	Lung		4×10^{-8}		10^{-8}	-
		GI (LLI)	3×10^{-3}		10^{-3}		-
$^{55}_{26}$Fe	(sol.)	Spleen	0.02	9×10^{-7}	8×10^{-3}	3×10^{-7}	10^{3}
	(insol.)	Lung		10^{-6}		3×10^{-7}	-
		GI (LLI)	0.07		0.02		-
$^{59}_{26}$Fe	(sol.)	GI (LLI)	2×10^{-3}		6×10^{-4}		-
		Spleen		10^{-7}		5×10^{-8}	20
	(insol.)	Lung		5×10^{-8}		2×10^{-8}	-
		GI (LLI)	2×10^{-3}		5×10^{-4}		-
$^{60}_{27}$Co	(sol.)	GI (LLI)	10^{-3}	3×10^{-7}	5×10^{-4}	10^{-7}	-
		Total body				10^{-7}	10
	(insol.)	Lung		9×10^{-9}		3×10^{-9}	-
		GI (LLI)	10^{-3}		3×10^{-4}		-
$^{59}_{28}$Ni	(sol.)	Bone	6×10^{-3}	5×10^{-7}	2×10^{-3}	2×10^{-7}	10^{3}
	(insol.)	Lung		8×10^{-7}		3×10^{-7}	-
		GI (LLI)	0.06		0.02		-

[a] The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

Radionuclide		Critical organ[a]	Maximum permissible concentrations For 40-h-week		For 168-h week		Maximum permissible burden in total body (μCi)
			(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	
$^{64}_{29}$Cu	(sol.)	GI (LLI)	0.01	2×10^{-6}	3×10^{-3}	7×10^{-7}	-
	(insol.)	GI (LLI)	6×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
$^{65}_{30}$Zn	(sol.)	Total body Prostate Liver	3×10^{-3}	10^{-7} 10^{-7} 10^{-7}	10^{-3} 10^{-3} 10^{-3}	4×10^{-8} 4×10^{-8}	60 70 80
	(insol.)	Lung GI (LLI)	5×10^{-3}	6×10^{-8}	2×10^{-3}	2×10^{-8}	- -
$^{69}_{30}$Zn	(sol.)	GI (S) Prostate	0.05	7×10^{-6}	0.02	2×10^{-6}	- 0.8
	(insol.)	GI (S)	0.05	9×10^{-6}	0.02	3×10^{-6}	-
$^{72}_{31}$Ga	(sol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	8×10^{-8}	-
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	6×10^{-8}	-
$^{71}_{32}$Ge	(sol.)	GI (LLI)	0.05	10^{-5}	0.02	4×10^{-6}	-
	(insol.)	Lung GI (LLI)	0.05	6×10^{-6}	0.02	2×10^{-6}	- -
$^{76}_{33}$As	(sol.)	GI (LLI)	6×10^{-4}	10^{-7}	2×10^{-4}	4×10^{-8}	-
	(insol.)	GI (LLI)	6×10^{-4}	10^{-7}	2×10^{-4}	3×10^{-8}	-
$^{75}_{34}$Se	(sol.)	Kidney Total body	9×10^{-3}	10^{-6} 10^{-6}	3×10^{-3} 3×10^{-3}	4×10^{-7}	90 100
	(insol.)	Lung GI (LLI)	8×10^{-3}	10^{-7}	3×10^{-3}	4×10^{-8}	-
$^{82}_{35}$Br	(sol.)	Total body GI (SI)	8×10^{-3} 8×10^{-3}	10^{-6}	3×10^{-3} 3×10^{-3}	4×10^{-7}	10 -
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	6×10^{-8}	-
$^{86}_{37}$Rb	(sol.)	Total body Pancreas Liver	2×10^{-3} 2×10^{-3}	3×10^{-7} 3×10^{-7}	7×10^{-4} 7×10^{-4}	10^{-7} 10^{-7} 10^{-7}	30 30 40
	(insol.)	Lung GI (LLI)	7×10^{-4}	7×10^{-8}	2×10^{-4}	2×10^{-8}	- -

[a] The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

Radionuclide		Critical organ [a]	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μCi)
			(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	
$^{87}_{37}$Rb	(sol.)	Pancrease	3×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7}	200
		Total body				2×10^{-7}	200
		Liver				2×10^{-7}	200
	(insol.)	Lung		7×10^{-8}		2×10^{-8}	-
		GI (LLI)	5×10^{-3}		2×10^{-3}		-
$^{85}_{38}$Sr	(sol.)	Total body	3×10^{-3}	2×10^{-7}	10^{-3}	8×10^{-8}	60
	(insol.)	Lung		10^{-7}		4×10^{-8}	-
		GI (LLI)	5×10^{-3}		2×10^{-3}		-
$^{89}_{38}$Sr	(sol.)	Bone	3×10^{-4}	3×10^{-8}	10^{-4}	10^{-8}	4
	(insol.)	Lung		4×10^{-8}		10^{-8}	-
		GI (LLI)	8×10^{-4}		3×10^{-4}		-
$^{90}_{38}$Sr	(sol.)	Bone	4×10^{-6}	3×10^{-10}	10^{-6}	10^{-10}	2
	(insol.)	Lung		5×10^{-9}		2×10^{-9}	-
		GI (LLI)	10^{-3}		4×10^{-4}		-
$^{90}_{39}$Y	(sol.)	GI (LLI)	6×10^{-4}	10^{-7}	2×10^{-4}	2×10^{-8}	-
	(insol.)	GI (LLI)	6×10^{-4}	10^{-7}	2×10^{-4}	3×10^{-8}	-
$^{91}_{39}$Y	(sol.)	GI (LLI)	8×10^{-4}		3×10^{-4}		-
		Bone		4×10^{-8}		10^{-8}	5
	(insol.)	Lung		3×10^{-8}		10^{-8}	-
		GI (LLI)	8×10^{-4}		3×10^{-4}		-
$^{95}_{40}$Zr	(sol.)	GI (LLI)	2×10^{-3}		6×10^{-4}		-
		Total body		10^{-7}		4×10^{-8}	20
	(insol.)	Lung		3×10^{-8}		10^{-8}	-
		GI (LLI)	2×10^{-3}		6×10^{-4}		-
$^{97}_{40}$Zr	(sol.)	GI (LLI)	5×10^{-4}	10^{-7}	2×10^{-4}	4×10^{-8}	-
	(insol.)	GI (LLI)	5×10^{-4}	9×10^{-8}	2×10^{-4}	3×10^{-8}	-
$^{95}_{41}$Nb	(sol.)	GI (LLI)	3×10^{-3}		10^{-3}	2×10^{-7}	-
		Total body		5×10^{-7}		2×10^{-7}	40
	(insol.)	Lung		10^{-7}		3×10^{-8}	-
		GI (LLI)	3×10^{-3}		10^{-3}		-

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Radionuclide		Critical organ[a]	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μCi)
			(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	
$^{99}_{42}$Mo	(sol.)	Kidney GI (LLI)	5×10^{-3}	7×10^{-7}	2×10^{-3} 2×10^{-3}	3×10^{-7}	8 -
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	7×10^{-8}	-
$^{96}_{43}$Tc	(sol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	5×10^{-4}	8×10^{-8}	-
$^{106}_{44}$Ru	(sol.)	GI (LLI)	4×10^{-4}	8×10^{-8}	10^{-4}	3×10^{-8}	-
	(insol.)	Lung GI (LLI)	 3×10^{-4}	6×10^{-9} 	 10^{-4}	2×10^{-9} 	- -
$^{105}_{45}$Rh	(sol.)	GI (LLI)	4×10^{-3}	8×10^{-7}	10^{-3}	3×10^{-7}	-
	(insol.)	GI (LLI)	3×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7}	-
$^{103}_{46}$Pd	(sol.)	GI (LLI) Kidney	0.01 	 10^{-6}	3×10^{-3} 	 5×10^{-7}	- 20
	(insol.)	Lung GI (LLI)	 8×10^{-3}	7×10^{-7} 	 3×10^{-3}	3×10^{-7} 	- -
$^{105}_{47}$Ag	(sol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-
	(insol.)	Lung GI (LLI)	 3×10^{-3}	8×10^{-8} 	 10^{-3}	 3×10^{-8}	- -
$^{111}_{47}$Ag	(sol.)	GI (LLI)	10^{-3}	3×10^{-7}	4×10^{-4}	10^{-7}	--
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	8×10^{-8}	-
$^{109}_{48}$Cd	(sol.)	GI (LLI) Liver Kidney	5×10^{-3} 	 5×10^{-8} 	2×10^{-3} 	 2×10^{-8} 2×10^{-8}	- 20 20
	(insol.)	Lung GI (LLI)	 5×10^{-3}	7×10^{-8} 	 2×10^{-3}	3×10^{-8} 	- -
$^{113}_{50}$Sn	(sol.)	GI (LLI) Bone	2×10^{-3} 	 4×10^{-7}	9×10^{-4} 	 10^{-7}	- 30
	(insol.)	Lung GI (LLI)	 2×10^{-3}	5×10^{-8} 	 8×10^{-4}	2×10^{-8} 	- -

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Radionuclide	Critical organ[a]	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μCi)
		$(MPC)_w$ (μCi/cm^3)	$(MPC)_a$ (μCi/cm^3)	$(MPC)_w$ (μCi/cm^3)	$(MPC)_a$ (μCi/cm^3)	
$^{125}_{51}$Sb (sol.)	GI (LLI) Lung Total body Bone	3×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7} 2×10^{-7} 2×10^{-7} 2×10^{-7}	- 40 60 70
(insol.)	Lung GI (LLI)	3×10^{-3}	3×10^{-8}	10^{-3}	9×10^{-9}	- -
$^{127}_{52}$Te (sol.)	GI (LLI)	8×10^{-3}	2×10^{-6}	3×10^{-3}	6×10^{-7}	-
(insol.)	GI (LLI)	5×10^{-3}	9×10^{-7}	2×10^{-3}	3×10^{-7}	-
$^{129}_{52}$Te (sol.)	GI (S)	0.02	5×10^{-6}	8×10^{-3}	2×10^{-6}	-
(insol.)	GI (ULI)	0.02	4×10^{-6}	8×10^{-3}	10^{-6}	-
$^{131}_{53}$I (sol.)	Thyroid	6×10^{-5}	9×10^{-9}	2×10^{-5}	3×10^{-9}	
(insol.)	GI (LLI) Lung	2×10^{-3}	3×10^{-7} 3×10^{-7}	6×10^{-4}	10^{-7} 10^{-7}	- -
$^{132}_{53}$I (sol.)	Thyroid	2×10^{-3}	2×10^{-7}	6×10^{-4}	8×10^{-8}	0.3
(insol.)	GI (ULI)	5×10^{-3}	9×10^{-7}	2×10^{-3}	3×10^{-7}	-
$^{133}_{53}$I (sol.)	Thyroid	2×10^{-4}	3×10^{-8}	7×10^{-5}	10^{-8}	0.3
(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	4×10^{-4}	7×10^{-8}	-
$^{134}_{53}$I (sol.)	Thyroid	4×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7}	0.2
(insol.)	GI (S)	0.02	3×10^{-6}	6×10^{-3}	10^{-6}	-
$^{135}_{53}$I (sol.)	Thyroid	7×10^{-4}	10^{-7}	2×10^{-4}	4×10^{-8}	0.3
(insol.)	GI (LLI)	2×10^{-3}	4×10^{-7}	7×10^{-4}	10^{-7}	-
$^{133}_{54}$Xe (submersion)	Total body		10^{-5}		3×10^{-6}	-
$^{135}_{54}$Xe (submersion)	Total body		4×10^{-6}		10^{-6}	-
$^{137}_{55}$Cs (sol.)	Total body Liver Spleen Muscle	4×10^{-4}	6×10^{-8}	2×10^{-4} 2×10^{-4} 2×10^{-4} 2×10^{-4}	2×10^{-8}	30 40 50 50
(insol.)	Lung GI (LLI)	10^{-3}	10^{-8}	4×10^{-4}	5×10^{-9}	- -

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Radionuclide		Critical organ[a]	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μCi)
			(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	
$^{140}_{56}$Ba	(sol.)	GI (LLI)	8×10^{-4}		3×10^{-4}		-
		Bone		10^{-7}		4×10^{-8}	4
	(insol.)	Lung		4×10^{-8}		10^{-8}	-
		GI (LLI)	7×10^{-4}		2×10^{-4}		
$^{140}_{57}$La	(sol.)	GI (LLI)	7×10^{-4}	2×10^{-7}	2×10^{-4}	5×10^{-8}	-
	(insol.)	GI (LLI)	7×10^{-4}	10^{-7}	2×10^{-4}	2×10^{-8}	-
$^{143}_{59}$Pr	(sol.)	GI (LLI)	10^{-3}	3×10^{-7}	5×10^{-4}	10^{-7}	-
	(insol.)	Lung		2×10^{-7}		6×10^{-8}	-
		GI (LLI)	10^{-3}		5×10^{-4}		-
$^{147}_{61}$Pm	(sol.)	GI (LLI)	6×10^{-3}		2×10^{-3}		-
		Bone		6×10^{-8}		2×10^{-8}	60
	(insol.)	Lung		10^{-7}		3×10^{-8}	-
		GI (LLI)	6×10^{-3}		2×10^{-3}		-
$^{151}_{62}$Sm	(sol.)	GI (LLI)	0.01		4×10^{-3}		-
		Bone		6×10^{-8}		2×10^{-8}	100
	(insol.)	Lung		10^{-7}		5×10^{-8}	-
		GI (LLI)	0.01		4×10^{-3}		-
$^{152}_{63}$Eu (9.2 h)	(sol.)	GI (LLI)	2×10^{-3}	4×10^{-7}	6×10^{-4}	10^{-7}	-
	(insol.)	GI (LLI)	2×10^{-3}	3×10^{-7}	6×10^{-4}	10^{-7}	-
$^{152}_{63}$Eu (13 y)	(sol.)	GI (LLI)	2×10^{-3}		8×10^{-4}		-
		Kidney		10^{-8}		4×10^{-9}	20
	(insol.)	Lung		2×10^{-8}		6×10^{-9}	-
		GI (LLI)	2×10^{-3}		8×10^{-4}		-
$^{154}_{63}$Eu	(sol.)	GI (LLI)	6×10^{-4}		2×10^{-4}		-
		Kidney		4×10^{-9}		10^{-9}	5
		Bone		4×10^{-9}		10^{-9}	5
	(insol.)	Lung		7×10^{-9}		2×10^{-9}	-
		GI (LLI)	6×10^{-4}		2×10^{-4}		

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Radionuclides		Critical organ[a]	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μCi)
			(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	
$^{166}_{67}$Ho	(sol.)	GI (LLI)	9×10^{-4}	2×10^{-7}	3×10^{-4}	7×10^{-8}	-
	(insol.)	GI (LLI)	9×10^{-4}	2×10^{-7}	3×10^{-4}	6×10^{-8}	-
$^{170}_{69}$Tm	(sol.)	GI (LLI)	10^{-3}		5×10^{-4}		-
		Bone		4×10^{-8}		10^{-8}	9
	(insol.)	Lung		3×10^{-8}		10^{-8}	-
		GI (LLI)	10^{-3}		5×10^{-4}		-
$^{177}_{71}$Lu	(sol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-
	(insol.)	GI (LLI)	3×10^{-3}	5×10^{-7}	10^{-3}	2×10^{-7}	-
		Lung				2×10^{-7}	-
$^{182}_{73}$Ta	(sol.)	GI (LLI)	10^{-3}		4×10^{-4}		-
		Liver		4×10^{-8}		10^{-8}	7
	(insol.)	Lung		2×10^{-8}		7×10^{-9}	-
		GI (LLI)	10^{-3}		4×10^{-4}		-
$^{181}_{74}$W	(sol.)	GI (LLI)	0.01	2×10^{-6}	4×10^{-3}	8×10^{-7}	-
	(insol.)	Lung		10^{-7}		4×10^{-8}	-
		GI (LLI)	0.01		3×10^{-3}		-
$^{183}_{75}$Re	(sol.)	GI (LLI)	0.02		6×10^{-3}		-
		Total body	0.02	3×10^{-6}		9×10^{-7}	80
	(insol.)	Lung		2×10^{-7}		5×10^{-8}	-
		GI (LLI)	8×10^{-3}		3×10^{-3}		-
$^{190}_{77}$Ir	(sol.)	GI (LLI)	6×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
	(insol.)	Lung		4×10^{-7}		10^{-7}	-
		GI (LLI)	5×10^{-3}		2×10^{-3}		-
$^{192}_{77}$Ir	(sol.)	GI (LLI)	10^{-3}		4×10^{-4}		-
		Kidney		10^{-7}		4×10^{-8}	6
		Spleen		10^{-7}			7
	(insol.)	Lung		3×10^{-8}		9×10^{-9}	-
		GI (LLI)	10^{-3}		4×10^{-4}		-

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Radionuclides		Critical organ [a]	Maximum permissible concentration For 40-h week (MPC)$_w$ (μCi/cm^3)	Maximum permissible concentration For 40-h week (MPC)$_a$ (μCi/cm^3)	For 168-h week (MPC)$_w$ (μCi/cm^3)	For 168-h week (MPC)$_a$ (μCi/cm^3)	Maximum permissible burden in total body (μCi)
$^{191}_{78}$Pt	(sol.)	GI (LLI)	4×10^{-3}	8×10^{-7}	10^{-3}	3×10^{-7}	-
	(insol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-
$^{193}_{78}$Pt	(sol.)	Kidney	0.03	10^{-6}	9×10^{-3}	4×10^{-7}	70
	(insol.)	Lung GI (LLI)	0.05	3×10^{-7}	0.02	10^{-7}	- -
$^{197}_{78}$Pt	(sol.)	GI (LLI)	4×10^{-3}	8×10^{-7}	10^{-3}	3×10^{-7}	-
	(insol.)	GI (LLI)	3×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-
$^{196}_{79}$Au	(sol.)	GI (LLI)	5×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
	(insol.)	Lung GI (LLI)	4×10^{-3}	6×10^{-7}	10^{-3}	2×10^{-7}	-- -
$^{198}_{79}$Au	(sol.)	GI (LLI)	2×10^{-3}	3×10^{-7}	5×10^{-4}	10^{-7}	-
	(insol.)	GI (LLI)	10^{-3}	2×10^{-7}	5×10^{-4}	8×10^{-8}	-
$^{199}_{79}$Au	(sol.)	GI (LLI)	5×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
	(insol.)	GI (LLI)	4×10^{-3}	8×10^{-7}	2×10^{-3}	3×10^{-7}	-
$^{197}_{80}$Hg	(sol.)	Kidney	9×10^{-3}	10^{-6}	3×10^{-3}	4×10^{-7}	4
	(insol.)	GI (LLI)	0.01	3×10^{-6}	5×10^{-3}	9×10^{-7}	-
$^{203}_{80}$Hg	(sol.)	Kidney	5×10^{-4}	7×10^{-8}	2×10^{-4}	2×10^{-8}	4
	(insol.)	Lung GI (LLI)	3×10^{-3}	10^{-7}	10^{-3}	4×10^{-8}	- -
$^{200}_{81}$Tl	(sol.)	GI (LLI)	0.01	3×10^{-6}	4×10^{-3}	9×10^{-7}	-
	(insol.)	GI (LLI)	7×10^{-3}	10^{-6}	2×10^{-3}	4×10^{-7}	-
$^{201}_{81}$Tl	(sol.)	GI (LLI)	9×10^{-3}	2×10^{-6}	3×10^{-3}	7×10^{-7}	-
	(insol.)	GI (LLI)	5×10^{-3}	9×10^{-7}	2×10^{-3}	3×10^{-7}	-
$^{202}_{81}$Tl	(sol.)	GI (LLI)	4×10^{-3}	8×10^{-7}	10^{-3}	3×10^{-7}	-
	(insol.)	Lung GI (LLI)	2×10^{-3}	2×10^{-7}	7×10^{-4}	8×10^{-8}	- -

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Radionuclides		Critical organ [a]	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μCi)
			(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	(MPC)$_w$ (μCi/cm^3)	(MPC)$_a$ (μCi/cm^3)	
$^{204}_{81}$Tl	(sol.)	GI (LLI)	3×10^{-3}		10^{-3}	2×10^{-7}	-
		Kidney		6×10^{-7}		2×10^{-7}	10
	(insol.)	Lung		3×10^{-8}		9×10^{-9}	-
		GI (LLI)	2×10^{-3}		6×10^{-4}		
$^{203}_{82}$Pb	(sol.)	GI (LLI)	0.01	3×10^{-6}	4×10^{-3}	9×10^{-7}	-
	(insol.)	GI (LLI)	0.01	2×10^{-6}	4×10^{-3}	6×10^{-7}	-
$^{210}_{82}$Pb	(sol.)	Kidney	4×10^{-6}	10^{-10}	10^{-6}	4×10^{-11}	0.4
		Total body	4×10^{-6}		10^{-6}		4
	(insol.)	Lung		2×10^{-10}		8×10^{-11}	-
		GI (LLI)	5×10^{-3}		2×10^{-3}		-
$^{210}_{84}$Po	(sol.)	Spleen	2×10^{-5}	5×10^{-10}	7×10^{-6}	2×10^{-10}	0.03
		Kidney	2×10^{-5}	5×10^{-10}		2×10^{-10}	0.04
	(insol.)	Lung		2×10^{-10}		7×10^{-11}	-
		GI (LLI)	8×10^{-4}		3×10^{-4}		-
$^{211}_{85}$At	(sol.)	Thyroid	5×10^{-5}	7×10^{-9}	2×10^{-5}	2×10^{-9}	0.02
		Ovary	5×10^{-5}	7×10^{-9}	2×10^{-5}		0.02
	(insol.)	Lung		3×10^{-8}		10^{-8}	-
		GI (ULI)	2×10^{-3}		7×10^{-4}		
$^{220}_{86}$Rn	*	Lung		3×10^{-7}*		10^{-7}*	-
$^{222}_{86}$Rn	*	Lung		3×10^{-8}*		10^{-8}*	-
$^{226}_{88}$Ra	(sol.)	Bone	4×10^{-7}	3×10^{-11}	10^{-7}	10^{-11}	0.1
	(insol.)	GI (LLI)	9×10^{-4}	2×10^{-7}	3×10^{-4}	6×10^{-8}	-
$^{227}_{89}$Ac	(sol.)	Bone	6×10^{-5}	2×10^{-12}	2×10^{-5}	8×10^{-13}	0.03
	(insol.)	Lung		3×10^{-11}		9×10^{-12}	-
		GI (LLI)	9×10^{-3}		3×10^{-3}		-

* The daughter elements of Rn^{220} and Rn^{222} are assumed to be present to the extent that they occur in unfiltered air. For all other isotopes the daughter elements are not considered as part of the intake; and, if present, they must be considered on the basis of the rules for mixtures.

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Radionuclide		Critical organ[a]	Maximum permissible concentrations For 40-h week		For 168-h week		Maximum permissible burden in total body (μCi)
			$(MPC)_w$ (μCi/cm^3)	$(MPC)_a$ (μCi/cm^3)	$(MPC)_w$ (μCi/cm^3)	$(MPC)_a$ (μCi/cm^3)	
$^{234}_{90}$Th	(sol.)	GI (LLI)	5×10^{-4}		2×10^{-4}		-
		Bone		6×10^{-8}		2×10^{-8}	4
	(insol.)	Lung		3×10^{-8}		10^{-8}	-
		GI (LLI)	5×10^{-4}		2×10^{-4}		-
$_{90}$Th-nat*	(sol.)	Bone	3×10^{-5}	2×10^{-12}*	10^{-5}	6×10^{-13}*	0.01
		Lung		4×10^{-12}		10^{-12}	-
		GI (LLI)	3×10^{-4}		10^{-4}		-
$^{233}_{92}$U	(sol.)	GI (LLI)	9×10^{-4}		3×10^{-4}		-
		Bone		5×10^{-10}		2×10^{-10}	0.05
	(insol.)	Lung		10^{-10}		4×10^{-11}	-
		GI (LLI)	9×10^{-4}		3×10^{-4}		-
$^{235}_{92}$U	(sol.)	GI (LLI)	8×10^{-4}		3×10^{-4}		-
		Kidney		5×10^{-10}		2×10^{-10}	0.03
		Bone				2×10^{-10}	0.06
	(insol.)	Lung		10^{-10}		4×10^{-11}	-
		GI (LLI)	8×10^{-4}		3×10^{-4}		-
$_{92}$U-nat	(sol.)	GI (LLI)	5×10^{-4}		2×10^{-4}		-
		Kidney		7×10^{-11}		3×10^{-11}	5×10^{-3}
	(insol.)	Lung		6×10^{-11}		2×10^{-11}	-
		GI (LLI)	5×10^{-4}		2×10^{-4}		
$^{239}_{94}$Pu	(sol.)	Bone	10^{-4}	2×10^{-12}	5×10^{-5}	6×10^{-13}	0.04
	(insol.)	Lung		4×10^{-11}		10^{-11}	-
		GI (LLI)	8×10^{-4}		3×10^{-4}		-
$^{241}_{95}$Am	(sol.)	Kidney	10^{-4}	6×10^{-12}	4×10^{-5}	2×10^{-12}	0.1
		Bone	10^{-4}	6×10^{-12}		2×10^{-12}	0.05
	(insol.)	Lung		10^{-10}		4×10^{-11}	-
		GI (LLI)	8×10^{-4}		3×10^{-4}		
$^{242}_{96}$Cm	(sol.)	GI (LLI)	7×10^{-4}		2×10^{-4}		-
		Liver		10^{-10}		4×10^{-11}	0.05
	(insol.)	Lung		2×10^{-10}		6×10^{-11}	-
		GI (LLI)	7×10^{-4}		2×10^{-4}		-

* Provisional values for Th-nat.

[a] The abbreviations GI, S, SI, ULI and LLI refer to gastrointestinal tract, stomach, small intestine, upper large intestine and lower large intestine, respectively.

APPENDIX IV

HOW TO PUT ON AND TAKE OFF RUBBER GLOVES[4]

The technique employed in this procedure is such that the inside of the glove is not touched by the outside, nor is any part of the outside allowed to come into contact with the bare skin.

The procedure is as follows:

(1) The gloves should be dusted internally with talcum powder.
(2) The cuff of each glove should be folded over, outwards, for about 5 cm.
(3) Put one glove on by grasping only the internal folded-back part with the other hand.
(4) Put the second glove on by holding it with the fingers of the gloved hand tucked in the fold and only touching the outside of the glove.
(5) Unfold the gloves by manipulating the fingers inside the fold.
(6) In taking off the gloves, seize the fingers of one glove by the other gloved hand and pull free.
(7) Take off the other glove by manipulating the fingers of the free hand under the cuff of the glove and fold it back so that an internal part is exposed which may be seized, and the remaining hand freed.

It is a great advantage if the inside and the outside of the gloves are distinctly different, e.g. in colour or texture.

APPENDIX V

RADIOACTIVE WASTE CONTROL AND DISPOSAL[5]

WASTE COLLECTION

In all working places where radioactive wastes may originate, suitable receptacles should be available.

Solid waste should be deposited in refuse bins with foot-operated lids. The bins should be lined with removable paper or plastic bags to facilitate removal of the waste without contamination.

Liquid waste should, if no other facilities for liquid waste disposal exist, be collected in bottles kept in pails or trays designed to retain all their contents in the event of breakage.

All receptacles for radioactive wastes should be clearly identified. In general, it will be desirable to classify radioactive wastes according to methods of disposal or of storage and to provide separate containers for the various classifications used. Depending upon the needs of the installation, one or more of the following bases for classification of wastes may be desirable:

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[4] Adapted from "Safety Techniques for Radioactive Tracers", Cambridge (1958).

[5] Adapted from the International Atomic Energy Agency Safety Series No. 1: Safe Handling of Radioisotopes (1962).

Gamma-radiation levels (high, low),
Total activity (high, intermediate, low),
Half-life (long, short),
Combustible, non-combustible.

For convenient and positive identification, it may be desirable to use both colour coding and wording.

Shielded containers should be used when necessary.

It is generally desirable to maintain an approximate record of quantities of radioactive wastes released to drainage systems, to sewers, or for burial. This may be particularly important in the case of long-lived radioisotopes. For this purpose it is desirable or necessary to maintain a record of estimated quantities of radioactivity deposited in various receptacles, particularly those receiving high levels of activity or long-lived isotopes. Depending upon the system of control used by the installation, it may be desirable to provide for the receptacle to be marked or tagged with a statement of its contents.

Radioactive wastes should be removed from working places by designated personnel under the supervision of the "radiological health and safety officer".

WASTE STORAGE

All wastes which cannot be immediately disposed of in conformity with the requirements of the competent authority have to be placed in suitable storage.

Storage may be temporary or indefinite. Temporary storage is used to allow for decrease of activity, to permit regulation of the rate of release, to permit monitoring of materials of unknown degree of hazard or to await the availability of suitable transport. Indefinite storage in special places has to be provided for the more hazardous wastes for which no ultimate disposal method is available to the particular user.

Storage conditions should meet the safety requirements for storage of sources.

The storage site should not be accessible to unauthorized personnel. (Control of animals should not be overlooked.)

The method of storage should prevent accidental release to the surroundings.

Appropriate records should be kept of the storage.

DISPOSAL OF WASTES TO THE ENVIRONMENT

General considerations

Disposal of radioactive wastes to the environment should be carried out in accordance with the conditions established by the "radiological health and safety officer" and by the competent authority.

The ways in which radioactive materials may affect the environment should be carefully examined for any proposed waste-disposal method.

The capacity of any route of disposal to accept wastes safely depends on the evaluation of a number of factors, many of which depend on the particular local situation. By assuming unfavourable conditions with respect to all factors, it is possible to set a permissible level for waste disposal which will be safe under all circumstances. This usually allows a very considerable safety factor. The real capacity of a particular route of waste disposal can only be found by a lengthy study by experts.

The small user should first try to work within restrictive limits which are accepted as being safe and which will usually provide a workable solution to the problem of waste disposal. Such a restrictive safe limit is provided by keeping the level of activity at the point of release into the environment below the permissible levels for non-occupationally exposed persons recommended by the International Commission on Radiological Protection for activity in drinking water or in air and indicated in Appendix III. This rule should be superseded if the competent authority provides any alternative requirements or if local studies by experts provide reasonable justification for other levels.

Disposal to drains and sewers

The release of wastes into drains does not usually need to be considered as a direct release into the environment. Hence, a restrictive safe limit will usually be provided if the concentrations of radioactive waste material based on the total available flow of water in the system, averaged over a moderate period (daily or monthly), would not exceed the maximum permissible levels for drinking water recommended by the International Commission on Radiological Protection for individuals occupationally exposed; these are indicated in Appendix III. This would provide a large safety factor since water from drains and sewers is not generally to be considered as drinking water. However, in situations where the contamination affects the public water supply, the final concentrations in the water supply should be to the levels set for non-occupationally exposed persons. Some present studies suggest that if the contamination affects water used for irrigation, the final concentrations in the irrigating water should be lower by a factor of at least ten below the levels set for occupational exposure and the possible build up of activity in the irrigated lands and crops should be carefully surveyed.

Finally, before release of wastes to public drains, sewers and rivers, the competent authorities should be informed and consulted to ascertain that no other radioactive release is carried out in such a way that the accumulation of releases will create a hazardous situation.

Radioactive wastes disposed to drains should be readily soluble or dispersible in water. Account should be taken of the possible changes of pH due to dilution, or other physico-chemical factors which may lead to precipitation or vaporization of diluted materials.

In general, the excreta of persons being treated by radioisotopes do not call for any special consideration. (This, however, does not apply to the unused residues of medical isotope shipments.)

Wastes should be flushed down by a copious stream of water.

The dilution of carrier-free material by the inactive element in the same chemical form is sometimes helpful.

Maintenance work on active drains within an establishment should only be carried out with the knowledge and under the supervision of the "radiological health and safety officer". Special care should be given to the possibility that small sources have been dropped into sinks and retained in traps or catchment basins.

The release of waste to sewers should be done in such a manner as not to require protective measures during maintenance work of the sewers outside the establishment, unless other agreement has been reached with the authority in charge of these sewers. The authority in charge of the sewer system outside the establishment should be informed of the release of radioactive wastes in this system; mutual discussion of the technical aspects of the waste disposal problem is desirable to provide protection without unnecessary anxiety.

Disposal to the atmosphere

Release of radioactive waste in the form of aerosols or gases into the atmosphere should conform with the requirements of the competent authority.

Subject to the competent authority, concentrations of radioactive gases or aerosols at the point of release into the environment should not exceed the accepted maximum permissible levels for non-occupationally exposed persons referred to in Appendix III. If higher levels are required and protection is based on an elevated release point from a stack, such levels can only be set after examination of local conditions by an expert.

Even if activity below permissible levels is achieved at the release point for an aerosol, a hazard or nuisance may still arise from fall-out of coarse particles. Therefore, the need for filtration should be assessed.

Used filters should be handled as solid wastes.

Burial of wastes

Burial of wastes in soil sometimes provides a measure of protection not found if the wastes are released directly into the environment. The possibilities of safe burial of waste should always be appraised by an expert.

Burial under a suitable depth of soil (about one metre) provides economical protection from the external radiation of the accumulated deposit.

A burial site should be under the control of the user with adequate means of excluding the public.

A record should be kept of disposals into the ground.

Incineration of wastes

If solid wastes are incinerated to reduce the bulk to manageable proportions, certain precautions should be taken.

The incineration of active wastes should only be carried out in equipment embodying those features of filtration and scrubbing as may be necessary for the levels of activity to be disposed of.

Residual ashes should be prevented from becoming a dust hazard, for example by damping them with water, and should be properly dealt with as ordinary active waste.

APPENDIX VI

DERIVATION OF EQUATION (42)

If the rate of exchange or "turnover" is proportional only to the amount of the substance present at any time, then the process is random and any single particle may have a life-time varying from 0 to ∞. The average life-time $(\bar{t})$ will be the sum of the existence times of all the particles divided by the initial number. Therefore, if R_0 represents the initial number of tracer particles present, then

$$\bar{t} = -\frac{1}{R_0} \int_{t=0}^{t=\infty} t \, dR \qquad (1)$$

However

$$R = R_0 e^{-kt} \qquad (2)$$

if the loss is by a first-order process

and

$$dR = -kR_0 e^{-kt} \, dt \qquad (3)$$

Substituting in (1)

$$\bar{t} = \frac{1}{R_0} \int_0^\infty tkR_0 e^{-kt} \, dt = k \int_0^\infty te^{-kt} \, dt \qquad (4)$$

Integrating

$$\bar{t} = K \left[-\frac{e}{k^2} (kt+1) \right]_0^\infty = k \left[0 + \frac{1}{k^2} \right] \qquad (5)$$

Therefore

$$\bar{t} = \frac{1}{k} \qquad (6)$$

and because

$$k = 0.693/T_{\frac{1}{2}B}$$

Therefore

$$\bar{t} = \frac{1}{0.693/T_{\frac{1}{2}B}} = 1.44 \; T_{\frac{1}{2}B} \qquad (7)$$

DESCRIPTION OF A THREE-COMPARTMENT MODEL

Consider the following open system as represented in Fig.29.

A model having one central compartment with peripheral compartments is generally termed a mamillary system. If compartment 1 is given a single bolus of tracer and then all compartments sampled as a function of time, the specific activity-time relationship may look as shown in Fig.30.

The specific activity in all three compartments would eventually reach the same value, indicating that the tracer bolus has mixed in the whole system. The continued decrease in specific activity can now be described by a single exponential k. The value 0.693/k can then be termed the biological half-life of the entire system. The curve a_1 can usually be resolved into a sum of exponentials (in the case of 3 compartments it would be a sum of 3 exponentials). Knowing by dilution the total exchangeable mass of the system, $S_1 + S_2 + S_3$, the five rate constants shown in Fig.1 can be estimated. The mathematics for such an exercise are beyond the scope of this discussion and the reader is referred to Refs [12] or [13] for solutions to such systems.

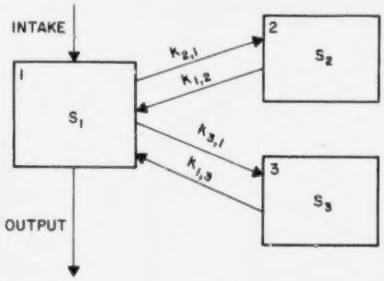

FIG. 29. A three-compartment open model (mamillary system).
$k_{1,2}$ = first-order rate constant describing exchange of tracer or tracee from compartment 2 to compartment 1, etc.

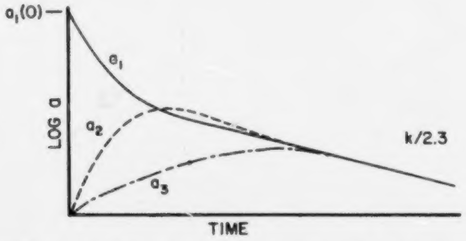

FIG. 30. Specific activity in compartments of a three-compartment open system with compartment 1 initially labelled.

a_1 = specific activity in compartment 1
a_2 = specific activity in compartment 2
a_3 = specific activity in compartment 3

APPLIED PART

INTRODUCTION

LABORATORY EXERCISES

INTRODUCTION

There can be little question that almost all laboratories engaged in animal research will soon utilize radionuclide techniques just as they now use calorimetry, spectrophotometry, chromatography, microscopy and other such procedures. This is due to the advantages of their use and is possible because of the availability of many radionuclides and that adequate instrumentation is rather inexpensive. In addition, experimental techniques have become highly developed and many research personnel have become highly proficient in this area. Often the use of radionuclides can provide experimental data that would otherwise be unobtainable. However, the design of such experiments must be on a sound scientific basis and include statistical considerations as well as those of radiological safety. Before attempting such experimentation the researcher must be acquainted with the principles of radionuclide methodology and obtain practice in special laboratory techniques and operation of counting instruments.

1. PRINCIPLES

The unique advantage of radionuclides is that their behaviour in a system is usually biologically identical to that of their counterpart and yet they can be identified easily by their characteristic radiation. The criteria for an ideal tracer is that it is indistinguishable from the tracee in biological behaviour and that its introduction does not disturb the system. Radionuclides uniquely fit these criteria since their chemical behaviour is identical to the stable counterpart and a great number of them can be produced in high specific activity. Since radiation can be detected with very high sensitivity generally negligible mass as well as activity need be administered to the system under study.

2. BASIC LIMITATIONS

There are several basic limitations inherent in tracer methodology which must be always considered. At first glance the listing of these may appear formidable indeed. However, in practice, with proper experimental design and knowledge of these limitations, reliable experimental data may be obtained.

2.1. Chemical effects

Most radionuclides are produced by methods which ensure a minimum of interfering chemicals. However, the investigator should be sure that the radionuclide preparation administered to the animal does not contain pyrogens, high concentrations of salts, high acidity or toxic metals.

The amount of carrier or tracee in a radionuclide preparation may be an important consideration. A classic example of the effect of mass on metabolic behaviour is the effect of stable iodide on the uptake of radioiodine by the thyroid. If kinetic response is to be measured then the tracer dose must be of high specific activity.

2.2. Radiochemical purity

It is very important in all tracer studies that the radionuclide solution does not contain other radionuclides than the one under study. Such extraneous radioactivity may arise from impurities in the target material or from the chemical separation processes used. Gamma-ray spectrometry, particularly using high-resolution detectors such as the semiconductor detectors, is an excellent method to check the primary solution for radioactive impurities. However, half-life of beta-adsorption determinations must be considered for pure beta emitters.

An important problem of chemical state arises when the carrier-free preparations are used, as the chemical behaviour at the extremely low concentrations that exist in carrier-free preparations may differ from that at ordinary concentrations. For example, the addition of a precipitating agent may not cause a solubility product to be exceeded at carrier-free concentrations. Also, the loss of minute amounts of reaction with trace impurities and absorption on walls of vessels or on suspended particles of dust and silica may be a sizeable fraction of the total at low concentrations, whereas, at the usual macroconcentrations these losses still occur but constitute a negligible fraction. In some solutions carrier-free tracers behave more like colloids than do solutes. Such particles of colloidal dimension containing radioactive atoms are called radiocolloids and these may behave rather differently in biological systems than do true solutes.

Another difficulty may arise due to the decay of radioactive atoms in secondary radioactive atoms usually termed "daughters". Such parent-daughter relationships must always be considered when the radiation being measured originates from both the parent and the daughter. This is particularly true of the naturally occurring radioactive materials which commonly have many radioactive daughters resulting from a long half-life parent. For example, see the decay scheme of ^{226}Ra.

2.3. Isotope effects

In most biological tracer work it is generally assumed that all isotopes of an element behave in an identical fashion. With the heavy elements this is generally a satisfactory assumption and any differences are certainly within experimental error. However, for the light elements, particularly hydrogen and carbon, it has been shown that biological fractionation of isotopes is termed the "isotope effect". There is much data documenting this effect in plants but relatively little concerning animals. Nevertheless, the investigator should be aware of such possibilities especially in processes dependent upon mass such as diffusion or reaction rates.

2.4. Radiation effects

It is essential that the experimental results should not be affected by any radiation received by the biological material and therefore the minimum amount of radioactivity should be used. This procedure also then minimizes the possibilities for personnel radiation exposure, for contamination of the laboratory and for problems with waste disposal. It is very important that the investigator carefully calculates the proper activity of radionuclide needed for each experiment. This will be based on factors such as the biological half-life and the counting yield of the detection instrument.

A factor of importance concerning biological effects of radiation is that the radionuclide may be incorporated into a critical biological molecule such as DNA where the radiation dose effect may be increased. Also, there may be chemical bond breaks by the recoil atom after disintegration or effects of transmutation. For example, consider a biological molecule that contains ^{32}P tracer. At the time of disintegration, the phosphorus atom in the molecule will be transformed into a sulphur atom and this may have an effect on the integrity or the function of the molecule. In practice this is rarely a limiting consideration due to the fact that the sensitivity of the method is so high that very small amounts of radioactivity are necessary. One may usually determine whether radiation effects are significant by performing replicate experiments using graded levels of radioactivity.

Another problem involving radiation effects is that of radiation decomposition. It is known that many ^{14}C-labelled organic compounds will undergo self-decomposition because of the radiation from the ^{14}C. This means that labelled organic compounds, especially those that are purchased commercially and may have been in storage for some time, should always be examined chromatographically before use to make sure there are no appreciable amounts of decomposition products present. Radiation decomposition can be reduced by several methods such as dissolution in a protecting solvent, adsorption on a solid matrix in thin layer, conversion to a stable derivative, and storage in the cold and in vacuum.

2.5. Exchange reactions

Isotopic exchange is a special case of exchange in which atoms in a given element interchange between two or more chemical forms of the element. In most metabolic studies, radiotracers are used to provide information on the processes involved in the biological synthesis of important metabolic compounds. If the radionuclide becomes incorporated in the metabolic product merely by exchange, which requires no energy production, then these experiments are of no value in that there is no evidence for synthesis of the product. Thus, it is necessary to carry out studies that can show whether or not exchange has occurred. This can be done by allowing the precursors and the product to react under conditions where the biological or energy producing system is interfered with. For example, animal tissues are known to convert inorganic phosphate into phospholipid. This is probably not an exchange process because experimental observations showed that (a) inorganic phosphate does not exchange with the phosphate radical or phospholipid when sodium phosphate is shaken with a phospholipid solution, (b) homogenized liver failed to form radioactive phospholipid from inorganic ^{32}P, and (c) respiratory inhibitors such as cyanide or carbon monoxide interfered with the formation of the tagged phospholipid.

It is also important to assure that there is no loss by exchange of the label from the molecule under study. For example, it would be of no avail to label the carboxyl hydrogen of an organic acid with tritium because as soon as this labelled acid were placed in the biological system the tritium would readily exchange with the hydrogen of the body water. On the other hand, a tritium label in an aldehyde group would be stable until the aldehyde was oxidized.

3. PRACTICE

3.1. Radionuclide preparation and delivery of dose

The investigator should have as much information as possible on the characteristics of the radionuclide preparation, particularly in regard to chemical or radiochemical impurities that may be present. It is necessary that the solution be suitable for administration to the animal from the standpoint of pH and freedom from particulate material. The investigator should confirm the identity of the radionuclide and radiochemical purity by determination of the half-life or energy spectrum. If dealing with a labelled organic material, it may be advisable to make a chromatographic evaluation to be sure that there are no decomposition products present.

In most studies it is necessary to administer the radionuclide to the animal quantitatively. If necessary, extensive practice should be undertaken with stable substances so that the investigator will be proficient before actually handling radioactive material. Standard methods can be used for oral, intravenous, subcutaneous, intramuscular and intraperitoneal injections. Inhalation doses are more difficult and require specialized procedures. It is usually necessary to prepare a standard solution. This should be done from the original preparation in such a way that the amount administered to the animal can be exactly correlated with the amount used to make up the standard solution. For example, this might involve using the same syringe or pipette for both processes.

3.2. Procedures with animals

It is always important that normal, healthy subjects should be used and should be so maintained during the study. Whenever possible the animal should be preconditioned to the type of handling and management that it is to receive during the experimental period. Needless to say, the scientist has a moral obligation to practice the highest degree of humaneness with respect to experimental animals. Such endeavours automatically improve the reliability of the results obtained.

After administration of radionuclides, the animal must be considered as a source of external radiation and of radioactive materials excreted and expired that can contaminate the surroundings. Provisions should be made for appropriate collection and disposition of radioactive excretions.

Reference: GAY, W.I. (Editor), Methods of Animal Experimentation, Academic Press (1965).

3.3. Handling of samples

The proper collection of samples is just as important as the reliability of subsequent analysis. It is important that the samples should be truly typical of the material that it represents.

In studies where the entire sample is not used, it is essential that proper mixing be done and that appropriate aliquots be taken. For example, care must be taken to mix faeces well before sampling, because successive increments may differ widely in activity especially at short times after administration of the radionuclide. The conventional grinding of dry material causes considerable difficulties with radionuclides because of the dust produced. Therefore, grinding is not recommended unless precautions are taken against hazards. In radionuclide studies cross-contamination must be particularly guarded against because of the extremely high dilutions that are often measured. Any radioactivity found at the site of administration may well have arrived there mechanically rather than metabolically. For example, after intraperitoneal injection, radioactivity found in the liver or other abdominal organs may have been deposited there mechanically. If the radionuclide has been given orally and the animal killed shortly thereafter, it is important that tissue samples not be allowed to come in contact with intestinal contents.

When fresh weights are necessary for concentration calculations on this basis the sample should be weighed before any appreciable moisture loss has occurred. With small samples such as adrenals, thyroids or pituitaries of laboratory animals, it becomes almost impossible to get accurate fresh weights and it is usually better to express results on a dry-weight basis. Depending on the experiments, samples should be dried as soon as possible after collection to minimize chemical and biological changes. Materials should be dried in a well-ventilated oven at 60-70° C, and if dry weights are required, the samples can be finished at 110° C.

Depending upon the characteristics and amount of the radionuclide present, fluid samples such as blood, plasma, urine, bile and milk may be assayed directly by liquid counting. With gamma-ray emitters it is possible to count solid samples directly. Where simultaneous chemical analyses or chemical separations are required it is frequently necessary to oxidize completely the organic matter of the sample. Depending upon the degree of completeness of ashing necessary, the following methods can be used: (a) Pseudo wet-ashing, which consists of dissolving small samples of tissue in concentrated nitric acid and making up to volume for liquid counting; (b) Conventional wet-ashing using Kjeldahl procedures or others that utilize oxidizing agents and various catalysts; (c) Conventional dry-ashing in the muffle furnace. With this procedure it is important to avoid losses due to volatilization, incorporation of the radionuclide into solid carbon particles, or adsorption onto the walls of the crucible; (d) Special oxidation procedures for ^{14}C or ^{3}H.

The form of the sample required for counting will be determined primarily by the energy or radiation emitted. In general, samples must be geometrically and physically uniform, these requirements being more rigorous for the lower-energy beta-emitters. The following procedures have been used for the preparation of solid samples from radioisotopes in solution or suspension: (a) Direct evaporation can be utilized. However, most biological samples will require a preliminary separation of unwanted soluble matter because of crystallization and creeping which results in non-uniform deposits. Direct evaporation is widely used with solutions such as calibration standards that have very little soluble material; (b) Many filtration devices have been developed for the purpose of quantitative collection of precipitates for radioassay. Techniques involving the use of filter paper suffer from the general disadvantages of variable texture, difficulty in reproducing constant weights, tendency of the paper and precipitate to buckle, and necessity for careful handling of the final sample to avoid damaging the precipitate; (c) Settling and centrifuging methods have been used to overcome many of the difficulties encountered in the filtration technique. Several simple devices can be used for this purpose;

(d) Electro-plating is an excellent method for the preparation of uniform thin films of many metals. Many types of electrolysis cells are available commercially for this purpose; (e) Dry powders may be pressed into shallow dishes with a spatula or may be formed into brickettes with a laboratory press and piston-cylinder apparatus.

The following exercises describe specific procedures. These are not necessarily the only methods or the best, but they have been found adequate under given conditions. It is hoped that the user of this manual will hereby have in his hands simple techniques to provide reliable data, and that he will be in a position to make such modifications as suit specialized needs.

LABORATORY EXERCISES

1. RADIOPHOSPHORUS DISTRIBUTION IN THE RAT AS DETERMINED BY TECHNIQUES OF WET-ASHING AND LIQUID COUNTING

Objectives

(1) To illustrate a convenient procedure for measurement of radioactivity in biological materials.
(2) To demonstrate a wet-ashing technique, a procedure for counting liquids, and methods of expression of data.
(3) To determine the distribution and turnover of ^{32}P in various tissues of the rat.

Introduction and theory

A great number of fundamental physiological and nutritional studies are based upon determination in various tissues or secretions of their content of a previously administered radioactive substance. The interest generally is in using a radiotracer to determine the behaviour of a normal body constituent. In this instance measurements are required of both the radionuclide and its stable counterpart in order to permit calculation of turnover rates. Or the interest may be in knowing the behaviour of the administered radionuclide itself, as for example in estimating retentions of radioactive environmental contaminants.

After entering the body, radioactive substances which are chemically identical with normal body constituents will generally be metabolized in exactly the same ways as their non-radioactive counterparts; both will have the same uptake and distribution. Thus, if equilibration has taken place, the amount of a radionuclide in a tissue will be related to the amount of stable element normally present. For example, a tissue which normally contains little phosphorus would be expected to take up relatively little radiophosphorus. This behaviour is quantitated by calculations and comparisons in terms of specific activities, which are expressed as amounts of radioactivity per amount of stable element. The behaviour of foreign substances may sometimes be related to that of chemical cogeners, e.g. strontium-calcium. However, the use of any such relationship should be based on experimental evidence as to the degree of interdependence that actually occurs.

The metabolic behaviour of substances in a biological system is typically studied by use of radiotracers according to the following scheme:

(1) The animal is acclimated to the experimental conditions.
(2) A known amount of the radiotracer is administered.
(3) Samples are collected after equilibration has been attained, or, for kinetic studies, after given time periods. These samples can be accessible materials such as blood, exhaled gases, milk, faeces or urine, which can be collected periodically, or they can be samples of tissues which require sacrificing the animal.
(4) The samples are converted into a physical state (usually identical and homogeneous) suitable for quantitative measurements of radioactivity. The treatment required depends in part upon the radiation characteristics of the nuclide.

(5) The radioactivity in each sample is measured, and if required, the stable nuclide is determined.

(6) The data are calculated to some standard base for purposes of comparison. This usually requires a standard calibration curve made from the radioactive preparation used for administration to the animal. Radioactivity is often expressed as "per cent of administered radioactive dosage per unit weight of tissue". Specific activity is often expressed as "per cent of radioactive dosage administered per unit weight of element". If it is known that the radionuclide is distributed through the animal in proportion to the body weight then the data can be normalized to a given body weight for comparison among animals.

Thus, almost every study of metabolism using radioactive tracers requires the accurate measurement of the amount of a radioactive material in a biological sample. In addition, it is usually necessary to measure the concentration of the stable element in order to calculate the turnover rate. One advantage of the use of radiotracers is the simplicity and convenience of the procedures. For radionuclides that emit gamma rays or beta particles of at least moderate energies, the production of a homogeneous and uniform sample suitable for radioassay can readily be accomplished by a wet-ashing procedure. If the sample can be measured directly in liquid state, then the overall procedure becomes one of considerable convenience.

Wet-ashing can be done by simple digestion in concentrated nitric acid. The resulting solution is then diluted to a given volume from which an aliquot is taken for the actual measurement. If the sample contains a large amount of fat, this can be extracted with iso-pentane alcohol which can be counted separately. The radioactivity values for each phase are added to give the total for the sample.

Materials

(1) Standard tray:
    (a) Porcelain tray (1).
    (b) 600-ml beaker marked WASTE (1).
    (c) 100-ml beaker marked DIST. H_2O (1).
    (d) 1-oz bottle of acetone.
    (e) Box of absorbent tissues (1).
    (f) Wax-coated dry-waste container (1).
    (g) Plastic squeeze bottle containing distilled H_2O (1).
    (h) Wax marking pencil (1).
    (i) Vacuum hose or other suitable arrangement for drying pipettes.
(2) Phosphate ^{32}P solution suitable for injection at pH ~6 (approx. 0.1 mCi/rat).
(3) G-M counter.
(4) Portable G-M survey meter.
(5) Mature rats (200-400g).
(6) Hot plate or Bunsen burner.
(7) Balance (accurate to 1 mg).
(8) Rat scale.
(9) Cages.
(10) Ether chamber.
(11) Concentrated nitric acid.

(12) 100-ml beakers (5).
(13) 500-ml volumetric flasks (2).
(14) 100-ml volumetric flasks (4).
(15) 100-ml measuring cylinder.
(16) 50-ml measuring cylinder.
(17) 25-ml measuring cylinder.
(18) 10-ml volumetric pipettes (2).
(19) 5-ml volumetric pipettes (3).
(20) 2-ml volumetric pipette.
(21) 1-ml volumetric pipette.
(22) 1-ml tuberculin syringe with 24-gauge 1.25-cm needle.
(23) Counting dishes (small Petri dishes will suffice).
(24) Planchets.
(25) Surgical scissors.
(26) Rat-tooth forceps.
(27) Infra-red heat lamp.
(28) Pipette control.

Procedure

(1) From the original radionuclide shipment prepare a dosing solution containing about 200 μCi ^{32}P/ml (solution A).
(2) Inject 0.5 ml of the dosing solution (solution A) intra-muscularly into the left hind leg of the rat. Leave the animal in a cage for about 1 h.
(3) Prepare dilutions of solution A for the calibration curve as follows:
  (a) Dilute 2 ml of solution A to 500 ml to give solution B.
  (b) Dilute 30 ml of solution B to 500 ml to give solution C. Note: Be sure that all dilutions are slightly acid to avoid loss of ^{32}P by adsorption on the glass of vessel walls.
  (c) Prepare the series of actual calibration solutions as follows:

Solution	Method of preparation
C	from step b
C/1.5	50 ml of solution C plus 25 ml H_2O
C/5	20 ml of solution C plus 80 ml H_2O
C/10	10 ml of solution C plus 90 ml H_2O
C/20	5 ml of solution C plus 95 ml H_2O

(4) Calibrate the G-M counter by counting 5-ml aliquots of each of the dilutions prepared in step 3(c) using the Petri dishes provided.
(5) At about 1 h after injection, kill the rat by placing in an ether chamber and then weigh the body.
(6) Remove small samples (0.5-1 g) of each of the following tissues: brain, liver, kidney, right femur, and gastrocnemius muscle. Avoid contamination of the tissue samples with radioactivity from the site of injection.

TABLE VII. EXAMPLE OF WORKING TABLE FOR EXPERIMENTS ON RADIOPHOSPHORUS DISTRIBUTION IN RATS

	A	B	C	D	E	F	G	H
Organ	Sample wt (g)	cpm in 5-ml aliquot	% of dose in 5-ml aliquot	% of dose in sample	% of dose per g of tissue	% of dose per g of tissue (normalized)	mg P/g of tissue	Specific activity % of dose/mg P (normalized)
Brain							2.5	
Muscle							2.0	
Kidney							3.0	
Liver							3.2	
Femur							100	

(7) Weigh each sample to the nearest milligram, place in a beaker, cover with concentrated nitric acid, and heat gently until the tissue is dissolved.

(8) Transfer quantitatively to a measuring cylinder, make up to 25 ml with water, mix, and pipette 5 ml into a sample dish for counting.

(9) Count the samples in exactly the same way that the calibration solutions were measured in step 4.

(10) Evaporate 1 ml of solution C to dryness in a counting planchet and count.

Calculations and reporting of results

(1) Record the following information in regard to the original ^{32}P preparation:
  (a) Amount of radioactivity;
  (b) Assay date and time;
  (c) Volume;
  (d) Specific activity;
  (e) Dilution factor used for preparation of solution A.
(2) Calculate microcuries administered to rat.
(3) Calculate mass of phosphorus administered to rat.
(4) (a) Record data on calibration curve as follows:

Solution	cmp (net)	μCi/ml	% of dose/5 ml
C			
C/1.5			
C/5			
C/10			
C/20			

  (b) Construct calibration curve on linear graph paper by plotting % of dose per 5 ml versus counts per minute.
  (c) Calculate a factor to convert observed counts per minute in 5 ml of solution to percentage of administered dose.
  (d) Compare the relative sensitivities of dry versus liquid counting.
(5) Complete Table VII to give results in terms of normalized tissue concentrations of ^{32}P and normalized specific activities.

Notes on calculations for Table VII:

(a) Values for column C (% of dose in 5-ml aliquot) can be derived directly from calibration or by use of factor computed in para. 4(c).

(b) Values for column D (% of dose in sample) are obtained by multiplication of column C value by the factor

$$\frac{\text{volume of total sample}}{\text{volume of counting aliquot}};$$

(c) Values for column E = $\dfrac{\text{value for column D}}{\text{value for column A}}$;

(d) Value for column F =

$$\text{Value for column E} \times \frac{\text{weight of rat used}}{\text{weight to which normalized (300 g)}};$$

(e) Values for column G (milligram P per gram of fresh tissues) represent typical data from literature;

(f) Values for column H = $\frac{\text{value for column F}}{\text{value for column G}}$

Questions

(1) Explain on a physiological basis why the amount of ^{32}P per unit weight of bone is relatively high and yet the specific activity is low.
(2) In this procedure, why is it usually not necessary to correct for coincidence losses or self-absorption? Under what conditions might it be necessary to do so? Why were you asked to establish a curve rather than just a single point from which the conversion factor could be calculated?
(3) Give an example of a study in which it would not be appropriate to normalize for body weights.
(4) List three radionuclides which could not be counted effectively by the method described in this exercise. Why?

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2. ERYTHROCYTE AND BLOOD-VOLUME MEASUREMENTS USING CHROMIUM-51-TAGGED ERYTHROCYTES

Objectives

(1) To illustrate a direct tracer dilution procedure.
(2) To estimate erythrocyte volume and total blood volume of a domestic or laboratory animal.

Introduction and theory

The earliest method for determination of the blood volume of an animal was proposed in 1854; it consisted of complete exsanguination and measurement of the blood collected. Since that time dilution techniques have been developed. In principle these consist of the following steps: (a) The administration of a "test-substance" or "tag" which is distributed through the volume to be measured: (b) The allowing of time for the administered substance to be uniformly distributed through this volume; and (c) The withdrawal of a small sample for the measurement of the concentration of the test substance. The amount of dilution which has occurred can then be used to calculate the total volume by which the test-substance was diluted.

Some of the earlier work was done using dyes, haemoglobin, carbon monoxide and other substances as tags or test substances. The availability of radionuclides provided tags with desirable characteristics such as ease of measurement, possibility of repeated measurements at relatively short time intervals, and uniform distribution through the volume to be measured.

For many years ^{32}P was widely used to tag red cells in order to determine erythrocyte volume. However, this technique has certain disadvantages such as the need to wash the cells after labelling which is time-consuming. Also, there is a fairly rapid loss of ^{32}P from the blood pool for which corrections must be made. As a matter of fact, the biological half-life of injected ^{32}P-labelled cells is estimated at 12-39 h. In addition, ^{32}P is a beta emitter and its radioassay is slightly more difficult than that for a gamma-ray emitter.

Radioactive iron (^{59}Fe) was used as early as 1940 and it has been shown that the tag remains with the erythrocytes during their entire life-span. However, iron released from breakdown of the red blood cells is available for further use in erythropoiesis.

In 1950, it was shown that radioactive chromium in the form of $Na_2{}^{51}CrO_4$ could be used to tag red blood cells, and this method has now become perhaps the most widely used for the determination of red blood cell volume and total blood volumes. Since ^{51}Cr is a gamma-ray emitter (0.32 MeV) it is easily counted in a well-type scintillation counter. Chromium-51 decays by electron capture. It can only be detected by its gamma ray which is emitted in only 9% of the disintegrations. The binding of the ^{51}Cr to the red cells is very stable; the ^{51}Cr is eluted from the red cells in the body with a half-life of around 80 d so that a much smaller correction factor for loss is necessary than with ^{32}P. In addition, the chromium released from the red cell on breakdown is not available for further use in erythropoiesis, and it is not necessary to wash the red blood cells after labelling since the chromium can be reduced chemically, in which case no further binding takes place.

The procedure, in general, consists of incubating a volume of the subject's blood in vitro with a suitable amount of radiochromium as the hexavalent chromate ion. At the end of the incubation period, ascorbic acid is added which reduces any unbound hexavalent chromium to the trivalent form thus rendering it unavailable for further binding.
A known volume is then injected into the animal and the activity of the injected suspension is measured in a well-type scintillation counter. At specified time intervals, a sample of the subject's blood is withdrawn and the activity determined.

The following symbols are used in calculations involving erythrocyte volume, plasma volume and blood volume:

V_{comp} = total volume of the compartment (red blood cells (V_c) or plasma (V_p);
V_i = volume of injected tag (red blood cells or plasma);
C_i = concentration (cpm/ml) of injected tag;
C_r = concentration (cpm/ml) of recovered tag;
V_b = total blood volume;
V_p = plasma volume;
V_c = red blood cell volume;

$V_{b(p)}$ = blood volume calculated from plasma volume;
$V_{b(c)}$ = blood volume calculated from red blood cell volume;
H_v = venous haematocrit (% cells);

H_m = mean body haematocrit = $\frac{V_c}{V_p + V_c}$;
F_c = F-cells factor;
$V_{c(p)}$ = red blood cell volume calculated from plasma volume;
$V_{p(c)}$ = plasma volume calculated from red blood cell volume.

From the general tracer dilution equation for a closed compartment system:

$$V_{comp} = V_i \left[\frac{C_i}{C_r} - 1\right] \qquad (1)$$

if V_{comp} is much greater than V_i, as in the study, then Eq. (1) becomes

$$V_{comp} = V_i \times \frac{C_i}{C_r} \qquad (2)$$

Equation (2) is used for calculating the erythrocyte volume. Plasma volume is determined in a similar manner with suitable corrections for removal of the trivalent chromium from the plasma.

After determining the plasma and erythrocyte volumes, the total blood volume may be determined by adding the two:

$$V_b = V_p + V_c \qquad (3)$$

When only the plasma volume or the red blood cell volume has been determined, the total blood volume may be calculated from either by employing the venous haematocrit value (% cells). Thus

$$V_{b(p)} = \frac{V_p}{(100 - H_v)} \times 100 \qquad (4)$$

and

$$V_{b(c)} = \frac{V_c}{H_v} \times 100 \qquad (5)$$

However, it has been shown that the venous haematocrit overestimates the mean body haematocrit. Therefore, plasma-haematocrit determinations of blood volume ($V_{b(p)}$) overestimate the true blood volume while the cell-haematocrit ($V_{b(c)}$) method underestimates the value. To compensate for these factors a factor (F_c) has been introduced which is defined as the ratio of mean body haematocrit to venous haematocrit:

$$F_c = \frac{H_m}{H_v} \tag{6}$$

Provided that the value for F_c is constant, Eqs (4) and (5) may be changed to:

$$V_{b(p)} = \frac{V_p \times 100}{100 - (H_v F_c)} \tag{7}$$

$$V_{b(c)} = \frac{V_c \times 100}{H_v F_c} \tag{8}$$

Finally, when only the plasma volume or the cell volume is determined experimentally, the following formulae can be used to calculate the other:

(a) If V_p is determined:

$$V_{c(p)} = V_p \times \frac{H_v}{100 - H_v} \tag{9}$$

(b) If V_c is determined:

$$V_{p(c)} = V_c \times \frac{100 - H_v}{H_v} \tag{10}$$

Materials and equipment

(1) Standard tray (see Exercise 1).
(2) ^{51}Cr as the hexavalent chromate.
(3) Well-type scintillation counter.
(4) Sheep in metabolism stalls.
(5) Centrifuge with head for 15-ml centrifuge tubes.
(6) Microcapillary centrifuge.
(7) Microcapillary haematocrit reader.
(8) Heparin solution (1000 I.U./ml).
(9) Citrate buffer (acetate-citrate-dextrose).
(10) Ascorbic acid (crystalline) pre-weighed to 120-mg samples.
(11) Saponin solution 0.1 g/litre.
(12) Na-chromate solution (0.1%).
(13) 20-ml syringes with 16- to 18-gauge needles (2).
(14) 10-ml syringes with 16- to 18-gauge needles (5).
(15) 2-ml syringe with a 20-gauge needle (1).
(16) 125-ml Erlenmeyer flasks (2).
(17) 100-ml volumetric flasks (2).
(18) 50-ml centrifuge tubes (4).

(19) 15-ml centrifuge tubes (4).
(20) 10-ml calibrated pipette.
(21) 5-ml volumetric pipettes (4).
(22) 2-ml volumetric pipettes (4).
(23) 1-ml volumetric pipettes (2).
(24) 0.5-ml Ostwald-Folin pipettes (10).
(25) Counting tubes (16).
(26) Capillary tubing.
(27) Bunsen burner.
(28) Pro-pipette.

Procedure

A. Tagging

(1) Withdraw approximately 20 ml of blood from the jugular vein of the animal with a heparin-wetted syringe and transfer to a 125-ml flask.
Note: if desired, the animals can be prepared by insertion of indwelling polyethylene cannulae in both jugular veins. In this instance one cannula can be used for withdrawal of blood and the other for injection. If dogs are used, the femoral vein is preferred; if rabbits are used, the ear vein.
(2) Add 8 ml of the citrate buffer solution to the sample of blood.
(3) Add an amount of the hexavalent ^{51}Cr solution containing approximately 100 μCi; record the time; swirl the suspension frequently and gently during the next 20 min.
(4) At the end of 20 min, add 120 mg of crystalline ascorbic acid and swirl the suspension for another 2 min.
(5) Determine, in triplicate, the haematocrit (H_v) of the erythrocytes in the suspension by the capillary-tube centrifugation method.

B. Injection and sampling

(1) Inject intravenously and quantitatively into the animal between 18 and 22 ml of the tagged suspension noting exactly the volume administered.
(2) Withdraw 10-ml blood samples from a vein (other than the one in which the suspension was injected) 5, 10, and 20 min after injection.

C. Preparation of standards and samples for counting

Standards

(1) Deliver 0.5 ml of the suspension into the saponin solution. Add 1 ml of a sodium chromate solution. Swirl gently and fill with water to the 100-ml mark. Make duplicate dilutions and count three 1-ml samples from each 100 ml.
(2) Place 5 ml of the suspension in a 15-ml centrifuge tube and spin into a 50-ml volumetric flask half filled with water. Add 2 ml of sodium chromate solution and fill to the 50-ml mark. Count three 1-ml samples.

Samples

(1) Determine the haematocrit (H_v) of erythrocytes in all samples in triplicate.
(2) Place 0.5 ml of blood in 2 ml of distilled water in counting tubes in duplicate.
(3) Collect the plasma from 5 ml of blood and place 0.5 ml in 2 ml of water for counting in duplicate.

Calculations and reporting of results

(1) Determine the net counts per minute per ml in the volume of suspension injected which are due to the erythrocytes present.
  (a) Volume of red blood cells reinjected.
  (b) Counts per minute per ml in red blood cells reinjected.
(2) Determine the concentration (cpm per ml of erythrocytes) in the samples taken and calculate the erythrocyte volume (V_p) using Eq. (2).

Time (min)	cpm/ml red blood cells	erythrocyte volume
5		
10		
20		

(3) Determine the concentration (cpm/ml) in the volume of plasma reinjected.
(4) Determine the concentration of plasma samples taken over the 20-min period and plot against time as the abscissa on semi-log paper.

Time (min)	cpm/ml plasma
5	
10	
20	

(5) Fix the best straight line by eye or method of least squares and extrapolate to zero time. What is the concentration at zero time?
(6) By using the concentration at zero time as (C_r), calculate the plasma volume (V_p) according to Eq. (2). What is the plasma volume?
(7) Determine the total blood volume ($V_c + V_p$) using V_p at zero time and V_c at 29 min. What is the total blood volume?
(8) Determine the F-cells factor. What is
  (a) H_v at 20 min,
  (b) H_m,
  (c) F_c?

Questions

(1) Why is it not necessary to wash the cells after incubating with ^{51}Cr, whereas it is necessary to wash them when ^{32}P is used?

(2) Why does the venous haematocrit overestimate the mean body haematocrit?
(3) Why must one extrapolate to zero time to determine the diluted concentration of ^{51}Cr in plasma?
(4) How would you determine the major routes of elimination of ^{51}Cr from the body?
(5) How should you monitor the laboratory for ^{51}Cr contamination?

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3. MEASUREMENT OF GASTRO-INTESTINAL BLOOD LOSS USING CHROMIUM-51-TAGGED ERYTHROCYTES

Objectives

(1) To illustrate the use of ^{51}Cr-labelled red cells for the measurement of blood loss into the gut.
(2) To compare the gastro-intestinal blood loss in a normal sheep with that in a parasitized sheep.

Introduction and theory

The labelling of red blood cells with ^{51}Cr is described in Exercise 2. A particularly useful property of erythrocytes tagged in this way is that, should blood loss occur into the gut, only a negligible proportion of the radionuclide is reabsorbed. This has been demonstrated in many species by the quantitative recovery in the faeces of doses of ^{51}Cr administered orally as ^{51}Cr-labelled cells. By comparing the total radioactivity of a complete 24-h collection of faeces with that of a blood sample taken at the beginning of the collection period, it is possible to estimate the amount of blood which has been lost from the circulation into the gut during that time. The method has been used to measure blood loss into the alimentary tract from bleeding ulcers and the blood loss into the gut brought about by certain parasitic worms in man and in animals.

The in-vivo stability of ^{51}Cr-labelled red cells is not the same for all species, and in sheep and cattle particularly there is a substantial loss of radionuclide from the red cells by elution over the first 24 h after injection. After the rapid elution phase there is a slower exponential loss of radionuclide from the circulation, but the slope of this disappearance curve is still much steeper than in other species. The somewhat abnormal behaviour of ^{51}Cr-tagged erythrocytes in sheep and cattle does not invalidate their use in the measurement of gastro-intestinal blood loss in that the radionuclide lost intravascularly is largely excreted in the urine. It is advisable, however, to make no measurements during the phase of rapid elution, i.e. for the first 48 h after injection.

Materials

(1) ^{51}Cr as hexavalent chromate.
(2) Well-type scintillation counter, preferably to take samples of at least 5 ml.
(3) Sheep: one normal (parasite-free), one experimentally infected with Fasciola hepatica at least eight weeks previously or one experimentally infected with Haemonchus contortus at least four weeks previously. Field cases of either parasitic infection may be used. The infected animals, from whatever source, should be showing some degree of anaemia. Male animals are preferred because by the use of a faecal bag and a metabolism stall complete collections of urine and faeces can be made without any fear of cross-contamination.
(4) Metabolism stalls for sheep.
(5) Animal clippers.
(6) Items for jugular contamination as described in Exercise 8.
(7) Microhaematocrit equipment as in Exercise 2.
(8) Laboratory centrifuge with buckets for 50-ml centrifuge tubes.
(9) Teat pipettes.

Procedure

A. Tagging

(1) Withdraw approximately 20 ml of blood from the jugular vein of the animal with a heparin-wetted syringe and transfer to a heparinized 50-ml centrifuge tube.
(2) Add an amount of hexavalent ^{51}Cr solution containing approximately 1 mCi and mix gently for 30 min.
(3) Centrifuge the sample and with a teat pipette draw off the supernatant and discard into container for radioactive liquid waste.
(4) Wash the labelled cells three times by filling up the tube with saline, mixing thoroughly, centrifuging and discarding the supernatant. Finally, make up the cell suspension with saline to approximately the original volume of blood. The labelled cells are washed in this exercise as it is important that the plasma should not contain radionuclides.

B. Injection and sampling

(1) The sheep should be given 2-3 days to become accustomed to the metabolism stalls and faecal bags before the experiment is started.
(2) Using the jugular catheterization technique described in Exercise 8, inject each animal with the total labelled erythrocytic suspension prepared from its own blood.

(3) 48 h after the injection, collect from the opposite jugular vein a 5-ml heparinized blood sample. Start the collection of urine and faeces.

(4) Each day at the same time remove a blood sample as above as well as the urine and faeces collected over the 24-h period. Continue this daily blood sampling and urine and faeces collection for a period of 10-14 days.

C. Preparation and counting of samples

(1) Pipette duplicate 1.0-ml lots of each blood sample into counting tubes and make up to the standard volume for counting (say, 5 ml) with approximately 0.01N NaOH. Larger volumes of blood may be used for counting later in the experiment when the concentration of ^{51}Cr particularly in the blood of the infected animal may be much reduced.

(2) On each blood sample carry out haematocrit determinations in triplicate.

(3) Weigh the total collection of faeces for each 24-h period, spread the pellets on a large sheet of paper, mix thoroughly and collect at random in counting tubes triplicate 5-g samples. With a glass rod pack these down to the appropriate volume for counting, e.g. 5 ml.

(4) With a graduated cylinder measure the volume of each urine collection and pipette out in duplicate suitable aliquots for counting.

(5) Using the well-type counter determine the net counts per minute of all the blood, urine and faeces samples. It is preferable that these should be done all together at the end of the experiment, but should it be necessary to measure the count-rates of samples from day to day as the experiment proceeds, they must all be corrected for decay and possible day-to-day variation in the sensitivity of the counting equipment by repeated measurements on one of the samples which is then treated as a standard.

Calculations and reporting of results

(1) From the cpm/ml for each blood sample and its haematocrit calculate the cpm/ml red blood cells.

(2) Plot cpm/ml red cells against time in days as abscissa on semi-log paper and fit the best straight line by eye or the method of least squares. Note the difference in slope of the lines obtained from the normal and the parasitized animals.

(3) For each 24-h period calculate a "faecal red-cell clearance" by dividing the total counts per minute of the 24-h collection of faeces by the cpm/ml red cells for the blood sample taken at the beginning of that period. This clearance represents the volume of red cells that would have to be lost into the gut each day to account for the ^{51}Cr activity of the faeces. Calculate the mean clearance figures for each animal over the period of the experiment and compare them.

(4) Calculate similarly clearance figures for the urine. These represent the volumes of red cells from which radionuclides are liberated each day within the body either by cell destruction or elution of radionuclides.

Questions

(1) What light do the above results throw on the aetiology of the anaemia in fascioliasis or haemonchosis?

(2) What inferences can be drawn about the nutrition of the parasites concerned?

(3) Why is it probably more accurate to express the loss into the gut in terms of red cells rather than whole blood?

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4. GASTRIC EXCRETION AND ABSORPTION OF MACROMOLECULES

Objectives

(1) To demonstrate an in-vivo technique for monitoring gastro-intestinal absorption and excretion.

(2) To observe the gastric excretion by the cat or dog of an injected foreign substance.

Introduction and theory

For pharmacological purposes it is often essential to know the biological fate of specific foreign substances. The sites of concentration, turnover rates, degree of degradation, and routes of removal may profoundly influence the usefulness of a given drug. For example, sulphasoxazole is the sulpha drug of choice for urinary infections because, after injection, it is rapidly excreted via the kidneys and hence high concentrations of the drug can be attained in the urinary tract. Neomycin is an excellent antibiotic for intestinal infections because it is not absorbed from the intestinal tract and hence high concentrations in the gut are possible after oral administration of the drug.

On the other hand, some drugs that have a definite therapeutic value may be contra-indicated because of deleterious side effects on the body as a whole or by action at certain sites. For example, sulphamethazine is a very good drug for intestinal infections but must be used with caution because of its tendency to precipitate in the nephron causing kidney stones. Polyvinylpyrrolidone (PVP) is an excellent blood-volume expander but is extremely dangerous because of its carcinogenic properties.

Thus, extensive studies must be made on any therapeutic or prophylactic preparation before it can be recommended for general use. Very often radiotracer techniques can be used to reduce the time and effort required to obtain the necessary information. In the present exercise the excretion into the digestive tract will be studied of either ^{14}C-labelled dextran or ^{131}I-labelled polyvinylpyrrolidone; both these macromolecular substances are used as blood-volume expanders.

The technique involves placing cannulae in each end of the stomach and collecting the stomach fluids at specified time intervals by flushing the stomach with a known amount of physiological saline solution and collecting the effluent. The volume of the gastric juice can be determined by difference, and the amount of radionuclide in the fluid calculated by measurement of the radioactivity in an aliquot using an appropriate detector.

Modifications of this procedure are widely used in studies of gastrointestinal absorption and secretion. In some instances when gamma-ray emitters are used the tagged element or compound is injected into a ligated intestinal segment and the amount remaining in the segment after a given time period is determined by merely removing the segment, placing it in a counting tube, counting, and comparing with a duplicate dose standard. Unidirectional fluxes can be calculated if the specific activity of the tagged substance in the gut is determined at the beginning and end of the time period. A more precise method of determining unidirectional fluxes is to perfuse a section of the intestinal tract at a known rate of flow with an automatic syringe filled with a solution of the test substance, collect the effluent, and calculate the fluxes from the amount of activity absorbed and the changes in concentration and specific activity that occur.

Materials

(1) Dogs or cats.
(2) ^{14}C-labelled dextran (50 μCi/animal) or ^{131}I-labelled polyvinylpyrrolidone[6] (PVP) (30 μCi/animal).
(3) Liquid scintillation counter or well-type scintillation counter.
(4) Scintillation solution

(a) PPO (2-5 diphenyloxazole)	10.0	g
(b) POPOP (1-4-bis-2-5-phenyloxazolyl)benzene	0.50	g
(c) Naphthalene	100.0	g
(d) Dioxane and Cellosolve[7] (1:1): make up to	1000	ml

(5) Na-pentobarbital (65 mg/ml).
(6) 0.9% NaCl solution (sterile).
(7) 30- to 50-ml syringe with a 16- to 18-gauge 2.5-cm needle.
(8) 10-ml syringes with new 20-gauge 2.5-cm needles (2).
(9) 5-ml syringe with new 20-gauge 2.5-cm needle.
(10) 100-ml-measuring cylinder.
(11) 100-ml beakers (12).
(12) Polyethylene cannulae (one to fit a 16- to 18-gauge needle).
(13) Liquid scintillation counting vials or counting tubes (15).
(14) General surgery kit including:
    (a) Scalpel
    (b) Surgical scissors
    (c) Haemostats (6)
    (d) Allis forceps
    (e) Thumb forceps
    (f) Needle holder
    (g) Half-round needle
    (h) Suture material (cotton thread).

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[6] Abbott Laboratories, Oak Ridge, Tenn., USA.

[7] Ethylene glycol mono-ethyl ether.

(15) Endotracheal tube.
(16) Operating table or board and ropes.
(17) Animal clippers or razor.
(18) Absorbent tissues.

Procedure

(1) Fast the experimental animal (dog or cat) for 12 or 24 h previous to the experiment.
(2) Anaesthetize the animal by the intravenous or intraperitoneal injection of Na-pentobarbital, administering about 1 ml per 2 kg body weight.
(3) If available, insert an endotracheal tube into the animal.
(4) Shave the abdomen with the clippers or a razor, and wash it with soap and water.
(5) Secure the animal on its back to an operating board or table.
(6) Incise the abdomen and present the stomach into the incision.
(7) Tie off the pylorus and insert a polyethylene cannula.
(8) Insert another cannula into the stomach at the cardia. This cannula should be of a diameter such that a 16- to 18-gauge needle will fit snugly into it.
(9) Replace the stomach as completely as possible into the peritoneal cavity with the cannulae extending from the incision. Cover the incision with saline-soaked absorbent tissues.
(10) Flush the stomach with saline to ensure a free flow of fluid through the organ.
(11) Slowly inject by the intravenous route about 50 μCi of the sterile ^{14}C-labelled dextran or 20-30 μCi of ^{131}I-labelled polyvinylpyrrolidone and note the time.
(12) At the end of 20 min flush the stomach from the cardiac end with 30-50 ml of isotonic saline solution and collect the effluent from the cannula in the pylorus. Empty the stomach as completely as possible and record the volume recovered.
(13) Repeat this procedure every 20 min for 4 h.
(14) If ^{14}C-dextran has been used pipette 3 ml of each flushing solution into a scintillation vial, add 15 ml of scintillation fluid and determine the ^{14}C content at the optimum high voltage setting in a liquid scintillation counter. If ^{131}I-labelled polyvinylpyrrolidone has been used, pipette 2 ml of each flushing solution into a counting tube and determine the radioactivity present in a well-type scintillation counter.

Calculations and reporting of results

(1) Plot the count rate per ml of gastric juice versus time as the abscissa. If it is impossible to determine the volume of gastric juice, plot the count rate per ml of flushing fluid versus time.
(2) Calculate the % dose secreted into the stomach during the course of the experiment.

Questions

(1) Is the radioactivity found in the stomach contents necessarily due to the presence of the macromolecule?
(2) If not, how can it be shown to be due to the molecule?
(3) How would you design an experiment to study the biological fate of chlortetracycline (aureomycin)?

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5. METABOLIC DISSOCIATION OF SHORT-LIVED BARIUM-137m FROM ITS CAESIUM-137 PARENT

Objectives

(1) To illustrate parent-daughter equilibrium relationships.
(2) To demonstrate the determination of the half-life of a short-lived radionuclide, ^{137m}Ba.
(3) To demonstrate the metabolic dissociation of barium from caesium in the animal body.

Introduction and theory

Caesium-137 has the decay scheme shown in Fig. 31.

In the animal body ^{137}Cs has a metabolic behaviour similar to that of potassium and accumulates primarily in soft tissues. ^{137m}Ba, however, behaves more like the other alkaline earths, calcium and strontium, and therefore becomes deposited primarily in bone. Thus, if an equilibrium mixture of ^{137}Cs-^{137m}Ba is introduced into the circulating fluids, there would be a preferential accumulation of caesium in soft tissues, whereas there would be a preferential deposition of the ^{137m}Ba in other tissues such as bone. This behaviour has been called metabolic dissociation. The movement of both caesium and its barium daughter into and out of a tissue stops when the blood supply to the tissue is interrupted. Following the interruption of the blood supply there would be a tendency for the radionuclides to attain the equilibrium mixture. That is, in tissues having an excess of caesium there would be growth of ^{137m}Ba until the equilibrium ^{137}Cs-^{137m}Ba level was attained. On the other hand, in tissues having an excess of ^{137m}Ba there would be a decay of the excess short-lived barium until the equilibrium was attained. From the growth or the disappearance of the ^{137m}Ba it is possible to calculate its half-life. Because of the short half-life of ^{137m}Ba it is necessary that accurate time records be kept during the experiment.

The metabolic dissociation of ^{137m}Ba from the parent ^{137}Cs is usually not observed experimentally because tissues are frequently not measured within minutes after removal from the organism; this delay permits the reestablishment of equilibrium conditions in the tissue samples (the rate of establishment of equilibrium is governed by the half-life of the daughter, 2.6 min).

The fact that there is an excess or deficiency of ^{137m}Ba in the tissues of the living animal which can be measured raises some interesting considerations: (1) Since the gamma-ray activities in tissues of the living animal are different from those expected from usual radioassay procedures, the radiation dosage may be different from that calculated from available ^{137}Cs levels. (2) ^{137m}Ba is produced within cells and the opportunity is presented for studying the mechanism by means of which this element is extruded from the cell. (3) Steady-state levels of ^{137m}Ba in clinical cases after ^{137}Cs administration may indicate both the state of the circulatory system and tissue metabolism; these factors, among others, should influence the level of excess ^{137m}Ba in the blood. (4) The excess ^{137m}Ba in the skeleton offers the possibility of studying the processes of rapid incorporation of alkaline earths into bone independent of the slower processes of growth.

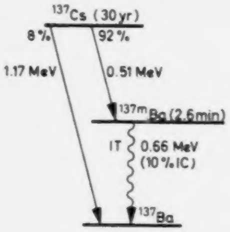

FIG. 31. Decay scheme of ^{137}Cs.

Materials

(1) ^{137}Cs- ^{137m}Ba (60 μCi/animal).
(2) Mature rats (250-300 g) fed or injected with the radionuclide several days before the experiment.
(3) Well-type scintillation counters, count-rate meters, and suitable chart recorders.
(4) Heparin (1000 I.U./ml).
(5) Sodium pentobarbital (6 mg/ml).
(6) 1-ml tuberculin syringes and 24-gauge 1.25-cm needles (2).
(7) 5-ml syringe and 20-gauge 2.5-cm needle.
(8) Dissection kit with haemostat.
(9) Wooden applicator sticks.
(10) Rat scale.
(11) Push-button timer or stop-watch.

Procedure

(1) Anaesthetize the rat by intraperitoneal injection of 1 ml of the solution of sodium pentobarbital per 100 g body weight.
(2) Clamp a haemostat over the tail at or near its base and start timer. This is zero time for this tissue. Cut a 1- to 2-cm piece from the middle of the tail. Drop the tissue into a scintillation tube and place the tube in the well as rapidly as possible.
(3) Start the recorder and adjust the count-rate meter so that the indicator reads just above the middle of the scale.
(4) As soon as the rate-meter is stabilized, note the time and mark the recorder sheet accordingly.
(5) Follow the decay until equilibrium is reached (about 15 min). Stop the recorder and mark the time.
(6) Open the abdominal cavity on the midline. Remove a 1-cm square piece of liver and start timer when cut is made. Follow the same sequence as with samples of tail in steps (2) to (5).
(7) Obtain blood by heart puncture (0.25 ml will suffice) and follow the same sequence as with samples of tail except that zero time is recorded when the first blood enters the syringe.

An alternate method can be employed in which a young chicken is used instead of the rat. In this instance convenient samples are blood and a small intestine 2 cm long.

Calculations and reporting of results

(1) Express the results as follows:
C = observed counts per minute per given sample.
C_{eq}= counts per minute per given sample observed at equilibrium.
(2) Using semi-log graph paper plot:
(a) C on the ordinate versus time on the abscissa.
(b) $C - C_{eq}$ on the ordinate versus time on the abscissa.
(3) From the slope of the $C - C_{eq}$ versus time curve, calculate the half-life of the ^{137m}Ba. Compare with the value reported in the literature.

Questions

(1) What physical characteristic determines the time required for a parent-daughter mixture to attain equilibrium composition? What is the approximate numerical relationship between this physical characteristic and the time required?
(2) Is an equilibrium composition attained if the half-life of the daughter is greater than that of the parent?
(3) What is the fundamental difference between "secular" and "transient" equilibrium?
(4) Explain how it is possible to study the metabolism of a radionuclide such as ^{137}Cs when the radioassay methods count the emissions of both the ^{137}Cs and the daughter ^{137m}Ba.
(5) List some advantages that result from the measurement of a daughter radioactivity in order to estimate the amount of the longer-lived parent present.

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6. DETERMINATION OF THYROID FUNCTION IN THE DOG BY EXTERNAL (IN VIVO) COUNTING AND MEASUREMENT OF PROTEIN-BOUND IODINE

Objectives

(1) To demonstrate external counting techniques including quantitative estimations, collimation, and scanning methods.
(2) To estimate thyroid function in the dog by measurement of the uptake of radioiodine by the thyroid gland and by determination of the protein-bound iodine (PBI) conversion ratio.

Introduction and theory

(1) General

Early chemical methods of estimating thyroid function were indirect, based on the administration of stable iodine and measurement of the amount excreted in the urine. If relatively small amounts were excreted, the patient was assumed to be hyperthyroid, if relatively large amounts, hypothyroid. The availability of radioiodine offers important advantages: it is possible to obtain a direct estimation by determination of the amount of radioiodine actually retained in the thyroid gland, using procedures that are simple and convenient.

As is well known, the thyroid gland preferentially accumulates inorganic iodine and converts it to a protein-bound form. If inorganic radioiodine is administered to an animal, it will behave exactly as does the stable form and will concentrate in the thyroid gland. Thus, the behaviour of the radioiodine provides an indication of the behaviour of normally existing inorganic iodine in the system. After administration of the ^{131}I, equilibrium is reached between the levels of the tracer in the plasma and the volume through which iodine diffuses rapidly. At this stage, the radioiodine is cleared mainly by the thyroid gland and kidney. Assuming that no significant changes in the rate of renal clearance occur in the absence of kidney disease, the thyroidal uptake of ^{131}I will reflect the degree of uptake of stable iodine and therefore is one of the parameters of thyroid function. The reasoning is that an overactive thyroid gland accumulates iodine faster and in greater amounts than does a normal gland. The reverse is true for an underactive thyroid gland. In dogs, for example, experiments indicate the following diagnostic ranges: 0 to 6% uptake, hypoactivity; 7 to 37% normal; greater than 38%, hyperactivity.

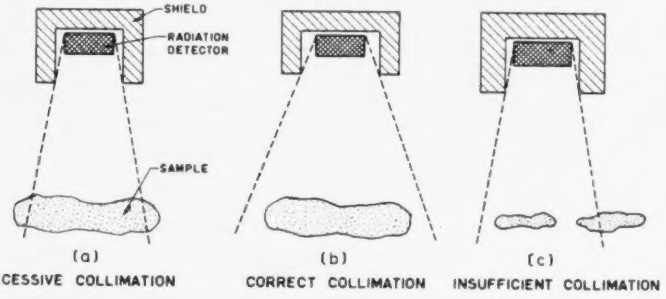
Since ^{131}I is a gamma-ray emitter (0.360 MeV), the amount of iodine present in the thyroid gland can be measured by properly placing a radiation detector at a specified distance from the skin of the animal in the region of the thyroid gland. A Geiger-Müller counter tube can be used. However, scintillation detectors are much more sensitive and are most commonly employed because they allow the measurements to be made with smaller doses of radioactivity. The external measurement on the animal is evaluated by comparison with the measurement of a dose standard in tissue-equivalent material counted at the same distance as in the animal and under identical conditions. The percentage uptake by the thyroid gland can then be determined as follows:

$$\%\text{ uptake} = \frac{\text{counts per minute of thyroid - background}}{\text{counts per minute of standard - background}} \times 100$$

Another parameter of thyroid function is the rate of conversion of inorganic radioiodine to the protein-bound form. The term "conversion ratio" (CR) is used to designate the per cent of radioiodine that has been converted to protein-bound iodine (PBI). The general procedure for this test is to obtain a blood sample at a specified time after injection of the ^{131}I, and then to pass the plasma through a special ion-exchange resin which removes any inorganic iodine. Radioassays are made of the original plasma and the effleunt to determine the ratio of counting rate in the effluent to that of the original plasma.

(2) Collimation

Difficulties in the measurement of radioactivity within the living animal are caused by the location of the radioactive material in the body, anatomical variations in the size and position of the organ containing the radioactivity, and especially variation in the concentration of activity both within the organ itself and in the surrounding tissues. It is usually necessary to use a collimated detector. The purpose of collimation is to exclude radiation from both general background and areas of the body other than the regions of interest. The amount of shielding required should be kept at a minimum in order to keep the bulk and mass of the detecting probe small enough to permit convenient handling. The shielding thickness of the collimator should be such that the radiation reaching the detector through the shielding is only a small fraction of that reaching the detector through the aperture. With ^{131}I usually 1-2 in. of lead are used. The principles of collimator use are illustrated in Fig.32.



(a) EXCESSIVE COLLIMATION (b) CORRECT COLLIMATION (c) INSUFFICIENT COLLIMATION

FIG. 32. Principles of collimator.

There are many factors involved that affect the absolute measurements. These include the size of the gland, the body background, the depth of the gland below the skin of the neck, and the degree of scattered radiation. In view of the complexity of the factors, determinations are usually made under arbitrary conditions that are rigorously controlled. The procedures described in this exercise represent typical methods.

(3) Scanning

It is often of value to know of the distribution of a radionuclide within the body. Such information can be obtained by a survey or scan which is made by systematically moving the radiation detecting instrument over the region of interest. This can be done by hand-held or standard mounted detectors. However, it is much more convenient to use an automatic scanning apparatus in which the radiation detector is moved mechanically in a pre-set pattern over the body. The basic equipment needed is a radiation detector with appropriate shielding and collimation, a mechanical system for moving the detector, and counting equipment for recording the information obtained.

Materials

(1) ^{131}I (approximately 50 μCi/dog of carrier-free ^{131}I in 1-2 ml).
(2) Dogs.
(3) Scintillation probe and stand with analytical count-rate meter.
(4) Well-type scintillation counter.
(5) Portable survey-meter.
(6) Automatic scintiscanner.
(7) Collimator sets and small lead filter for scintillation probe.
(8) Animal clippers.
(9) Centrifuge.
(10) Sodium pentobarbital (65 mg/ml).
(11) Heparin (1000 I.U./ml).
(12) Physiological saline solution.
(13) Counting tube with cotton for duplicate dose standard.
(14) Counting tubes (2).
(15) 10-ml syringe and 20-gauge 2.5-cm needle.
(16) 5-ml syringe and 20-gauge 2.5-cm needle.
(17) 2-ml syringe and 20-gauge 2.5-cm needle.
(18) 5-ml volumetric pipette.
(19) 2-ml volumetric pipette.
(20) 1-ml volumetric pipette.
(21) Operation boards and ropes.
(22) Scalpel, haemostats, forceps, scissors.
(23) Lead shield (a lead brick will suffice).
(24) 30-cm ruler.
(25) Thyroid phantom - a commercial plastic phantom may be used. If this is not available, a 1000-ml beaker filled with water may be used with the duplicate dose standard clamped in position in the beaker about 0.5-1.0 cm from the wall.

(26) Circles of filter paper.[8]
(27) Plastic ion-exchange column.[8]
(28) Circles of filter paper.[8]
(29) Small rubber bulb.[8]
(30) Plastic counting tubes.
(31) Pipette control.

Procedure

Note: The procedures as used here are more complex than needed for routine clinical diagnosis in order to provide additional information. For routine work it would not be necessary to anaesthetize the animal nor to remove its thyroid for comparative measurements. With well-behaved animals the measurements can easily be made without any extraordinary restraints.

(1) Inject intraperitoneally or intravenously into the dog about 50 μCi of ^{131}I. By means of the same syringe and procedure deliver an identical volume into a test-tube containing a wad of absorbent cotton; this is to serve as the standard. After 24 h proceed as follows to measure thyroidal uptake.
(2) Place the count-rate meter in operation.
(3) Place the heavy lead shield in front of the detector and determine the background.
(4) Anaesthetize the dog by intravenous injection of pentobarbital. Survey the area over the thyroid gland with a portable survey-meter to get an idea of the activity present.
(5) If a scintiscanner is available, make a scintigram of the thyroid gland.
(6) Place the dog on the board provided on its back and with the head extended by means of a jaw-holding attachment.
(7) With the detector exactly 30 cm above the thyroid gland, and with the small lead filter in place in the probe, take a maximum reading.
(8) Place the heavy lead shield (lead brick) over the gland and take another reading (this will be the background). Remove the dog from the detector.
(9) Take the duplicate dose standard in a tissue-equivalent phantom and place it at exactly the same distance from the detector as was the thyroid gland. Repeat the measurement of standard and background as was done on the dog.
(10) Sacrifice the animal with an over-dose of Na-pentobarbital, carefully dissect out the thyroid gland and place in a test tube. Place the carcass of the dog back in position and repeat the background measurement.
(11) Place the test tube containing the thyroid gland in the phantom and line it up with the detector at a distance that will give a reasonable count. Measure the activity.
(12) Replace the test tube with a tube containing the duplicate dose standard and repeat the count.
(13) Remove both samples from the vicinity and measure the background.

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[8] Obtainable as Ioresin PBI Conversion Ratio Kit from Abbott Laboratories, Oak Ridge, Tenn., USA.

(14) By means of either the scintiscanner or a scintillation probe and a suitable grid, determine the radiation patterns "seen" by the different collimators available. (The source used can be a mock ^{131}I source or the duplicate dose standard.)

(15) Determine the conversion to protein-bound iodine according to the following directions using the Abbott Kit:
   (a) Place a piece of the pre-cut filter paper on the bottom of the polyethylene cylinder.
   (b) Transfer to this cylinder, gently with a dropper, sufficient ion-exchange resin to produce a column 1.5 - 2 cm in height, and allow the water to drain through.
   (c) Wash the column once with physiological saline, allow it to drain, and express the excess liquid by gentle air pressure from the small rubber bulb supplied. (Care should be taken to break the seal between the bulb and the cylinder by turning and lifting gently, before releasing the pressure, so as not to disturb the resin column.)
   (d) Obtain some plasma and pipette 2 ml into a graduated test tube; measure the radioactivity in a well-type scintillation counter.
   (e) After counting, pour the sample of plasma onto the column and allow it to drain through.
   (f) When plasma has stopped dripping from the column, pressure is again applied with the bulb and the test tube is then washed with 2 ml of physiological saline followed by a second 1-ml wash. These washings are added to the top of the column and collected with the plasma effluent.
   (g) Using 2 ml of plasma, the volume will now be about 5 ml as indicated by the test-tube calibrations. This tube contains protein-bound ^{131}I only since the inorganic iodide has been retained by the resin column.
   (h) Count the liquid in a well-type scintillation counter as above.

Calculations and reporting of results

(1) Calculate the % of thyroidal uptake of ^{131}I from both in-vivo and in-vitro measurements.
(2) Determine the conversion of protein-bound iodine and express as the conversion ratio.

Questions

(1) List as many factors as you can that might be expected to alter the thyroidal retention of administered radioiodine.
(2) Scintillation detectors as used for external counting are very sensitive to low-energy scattered radiation. In what two ways can this source of error be eliminated?

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7. LIVER FUNCTION AS STUDIED WITH IODINE-131-LABELLED ROSE BENGAL

Objectives

(1) To illustrate a procedure for external counting of the liver of sheep.

(2) To estimate liver function in the normal and diseased animal by measurement of the uptake and excretion from the liver of ^{131}I-labelled Rose Bengal.

Introduction and theory

The metabolic behaviour of Rose Bengal, which is the potassium salt of tetraiodo-tetrachlor-fluorescein, has been used for many years to test liver function. When this dye is injected into the blood it is accumulated by the hepatic cells and excreted in the bile. Since the Kupffer cells of the reticuloendothelial system are not involved, the uptake by the liver of Rose Bengal is a measure of liver-cell function. There is no appreciable resorption of this dye from the bowel or biliary system and it leaves the body with the faeces. Urinary elimination is minimal except in cases of severe hepatic insufficiency.

In principle, three types of information are available from the use of this substance: (1) The extent of liver-cell function; (2) The patency or lack of obstruction of the bile duct; (3) The degree of hepatic circulation.

In earlier tests the concentration of the dye was measured colorimetrically and it was possible to measure hepatic cell function indirectly by the decrease of concentration in the blood as the serum was cleared of the dye by the liver. This procedure was extremely difficult and limited.

The chemical structure of Rose Bengal permits the replacement of the stable iodine atoms with radioactive iodine (^{131}I) by means of an ion-exchange reaction giving a high specific activity (1 mCi/mg) in the dye. When this radioactive Rose Bengal (^{131}RBI) is used the test has certain advantages over that based on colorimetric estimations. Important features are as follows:

(1) Hepatic-cell function is measured directly rather than by changes in blood. Thus, repeated venipunctures are eliminated and greater sensitivity is obtainable.

(2) The pattern of dye uptake and release from the liver is continuously recorded.

(3) The test can be used safely in the presence of bilary tract obstruction because of the minute quantities of dye needed, which eliminates the problem of toxicity.
(4) The patency of the biliary tract can be measured.

The procedure, in general, involves the detection and recording of the uptake and elimination of ^{131}I-labelled Rose Bengal by an appropriately positioned scintillation probe and count-rate meter connected to a chart recorder. The tagged dye accumulates rapidly in the liver, reaches a peak, which represents a transitory steady-state between rates of uptake and excretion, and then disappears from the liver comparatively slowly as the rate of excretion dominates. The downward slope is a measure of the rate at which the liver is capable of excreting the dye since the blood is essentially cleared by this time and there is no appreciable further uptake by the liver.

With liver pathology a variety of abnormalities of the graphic picture may be seen when a chart is compared with the record of a normal animal. A few of these are: decreased rate of uptake; decreased total uptake; decreased rate of removal; no removal. The test will be performed on two sheep one of which is normal, the other having a liver that is poisoned with carbon tetrachloride.

Recently, it has been shown that certain substances, when injected into an animal, can make the ^{131}RBI test more sensitive. One of these compounds is bromsulphthalein (BSP). If BSP is injected into an animal at a dose of 2.5 mg per kg of body weight just previous to the dose of ^{131}RBI, the BSP decreases the hepatic uptake of the labelled dye. By employing this technique on a number of patients, physicians have found that many people with liver pathology show an abnormally low uptake, whereas if given the labelled dye without the BSP the same subjects show a low normal uptake pattern. Individuals with normal livers show no decrease in uptake of Rose Bengal when given the same dose of BSP.

Materials

(1) ^{131}I-labelled Rose Bengal (25 μCi/animal).
(2) Sheep; one normal, one poisoned with CCl_4 (0.5ml/kg body wt by stomach tube 24 h previous to experiment).
(3) Scintillation probe and stand with analytical count-rate meter attached to chart recorder.
(4) Portable survey-meter.
(5) Metabolism stalls for sheep.
(6) Animal clippers.
(7) Stable Rose Bengal.
(8) Physiological saline solution.
(9) Heparin solution (100 I.U./ml).
(10) 5-ml syringes with 20-gauge 2.5-cm needle (2).
(11) Polyethylene tubing (PE 90).
(12) 15-gauge 2.5-cm needle.
(13) Haemostat.
(14) Adhesive tape.

Procedure

(1) Confine the animals in the metabolism stalls restrained so as to minimize lateral motion during counting.
(2) Shave the lateral side of the neck with the clippers and with the 15-gauge 2.5-cm needle make a venipuncture of the jugular vein.
(3) Slide a piece of the PE 90, previously filled with heparin, through the 15-gauge needle into the vein. Remove the needle leaving the piece of tubing in place as a cannula. Fasten it to the side of the neck with adhesive tape.
(4) Fill the cannula with the heparin solution through a 20-gauge needle and clamp off the cannula with a haemostat.
(5) Assemble the scintillation probe, lead shield, count-rate meter and recorder. The recorder should run at 6 in./h.
(6) Position the probe at a point on a horizontal line even with and 8.0 cm caudal to the point of the elbow. This position corresponds to the maximum concentration of liver tissue and is sufficiently distant from the gall-bladder area to avoid interference from labelled dye which becomes concentrated in the gall-bladder.
(7) Record background for 5 min using the 1000 scale and a 10-sec time constant.
(8) Switch to the 30 000 scale and 0.5-sec time constant.
(9) Inject 25 μCi ^{131}I-labelled Rose Bengal via the previously inserted polyethylene jugular cannula. Mix the labelled dye with sufficient stable Rose Bengal to make 2.5 - 3.0 ml; this tends to prolong the excretion time. Inject 5 ml of sterile physiological saline solution after the Rose Bengal to ensure that all the Rose Bengal has reached the blood stream.
(10) After 5 min, switch to the 10-sec time constant.
(11) Continue recording for $1\frac{1}{2}$ - 2h.
(12) At the end of 2 h and again at 24 h take readings over the heart, liver and abdomen.
(13) Determine activity of faeces at 24 h with a survey-meter.

Calculations and reporting of results

(1) Calculate the mean rate of uptake of the labelled Rose Bengal by the liver of the two sheep assuming that the peak represents 100% uptake.
(2) By making the same assumption, determine the rate of removal of the dye from the liver.
(3) With the data from (2), determine the time for about 95% of the dye to be removed from the liver.

Questions

(1) Why is collimation necessary in this procedure?
(2) What differences did you see in the uptake and excretion graphs of the two sheep?
(3) Can you explain these differences on a pathological basis?
(4) Why was a dose standard necessary in the thyroid function study but not important in this exercise?

(5) What type of graph would have been seen if one of the sheep had had an obstruction of the common bile duct?

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8. TRANSPORT OF CALCIUM-45 AND STRONTIUM-85 ACROSS SURVIVING INTESTINAL SEGMENTS

Objectives

(1) To demonstrate the use of the surviving intestinal sac.
(2) To illustrate an application of double counting techniques.
(3) To study the transport of calcium and strontium in relationship to mechanisms of gastro-intestinal absorption.

Introduction and theory

The everted sac

An important technique for the study of transport of substances across intestinal membranes is the use of surviving everted intestinal sacs from laboratory animals. After eversion, the mucosal side of the intestine is on the outside (see Fig. 33) and, when placed in contact with the incubating solution, it can be oxygenated to maintain mucosal function. The procedure is generally as follows. The animal is killed and an intestinal segment 6 - 8 cm long is immediately removed. The segment is washed with isotonic saline, everted, rewashed with the saline, and tied at one end with a suture.

The solution to be studied is injected into the other end through a ligature and this end is tied after removal of the needle. The filled segment is then placed in a flask and covered completely by incubating medium. The rate and extent of transport of material across the sac can be determined by analysis of the incubating medium and the sac contents at different times. If with time differing concentrations are found to develop within and without the sac this is evidence that the membrane was able to transport the radionuclide against a concentration gradient. The substance studied can be either labelled or unlabelled material. The studies can be done by starting with the same concentrations of substances on both sides of the membrane and observing if differing concentrations develop. Or the label can be placed only on one side and observations made as to the rate of movement and the final equilibrium levels attained.

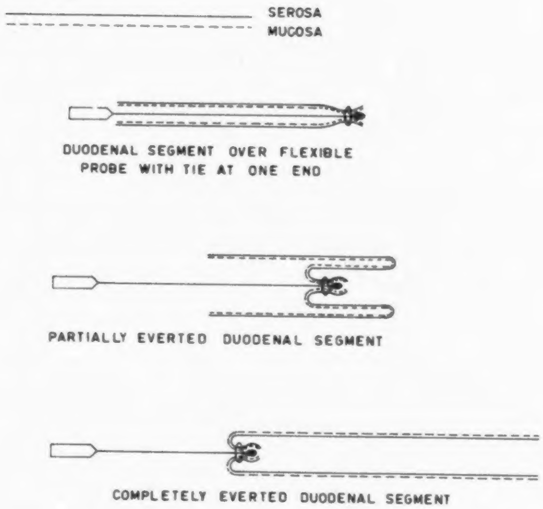
SEROSA
MUCOSA
DUODENAL SEGMENT OVER FLEXIBLE PROBE WITH TIE AT ONE END
PARTIALLY EVERTED DUODENAL SEGMENT
COMPLETELY EVERTED DUODENAL SEGMENT

FIG. 33. Eversion of a duodenal segment with the aid of a flexible probe.

The advantage of this system is that it permits precise measurements under restricted and specific experimental conditions. The disadvantage is, of course, that the system may not represent the situation as it exists in the living animal. However, there has been excellent agreement in several studies that have been done where it has been possible to make a comparison between the results obtained using surviving intestinal segments and preparations in the living animal.

Multiple tracer techniques

In this type of study as well as in many other biological experiments, there is considerable advantage in the simultaneous use of two radionuclides. This procedure requires accurate measurement of each when both are present in a single sample. There are various methods both physical and chemical that can be used for this purpose.

Spectrometry

If the two radionuclides emit different types of radiation (e. g. one a beta emitter and the other an EC emitter with an abundant gamma ray) they can be differentiated by counters that respond primarily to one of the radiation types. In this experiment, for example, ^{45}Ca emits only beta particles and ^{85}Sr decays by EC and emits only gamma rays. Therefore, a G-M counter may be used to measure the ^{45}Ca and a well-type NaI(Tl) scintillation counter to measure the ^{85}Sr.

If the two radionuclides both emit gamma rays, then gamma-ray spectrometry may be used to differentiate the two. Gamma-ray detectors with good-energy resolution, such as the semi-conductors, are particularly useful if the gamma-ray energies are close together. Alpha and beta spectrometry is also possible with semi-conductor detectors.

In the liquid scintillation counter differential beta-particle counting is possible if the beta energies differ by at least a factor of 4.

Chemical or physical separation

With isotopes of different elements, conventional physical or chemical separation may be employed. For example, the separation of ^{45}Ca from ^{32}P by precipitation of calcium oxalate. Ion-exchange columns are often useful for this purpose. In the use of ^{24}Na and ^{42}K in studies of body electrolytes it is possible to make simultaneous measurements in plasma by precipitating the potassium as cobaltinitrite. In studies of blood volume, where ^{131}I-labelled albumin and ^{51}Cr-labelled red cells are used, it is possible to separate these two radionuclides simply be centrifugal deposition of the red cells in the usual way. In multicounting methods, it is important to have the concentration of each radionuclide such that large errors are not introduced because of subtractive procedures for the particular method used. Theoretical considerations are quite complex for the optimizing of the methods and the concentrations of radionuclides. The bibliography should be consulted for details. It is emphasized that unless care is taken, it is quite likely that large uncertainties will be introduced in double-counting procedures.

Materials

(1) Standard tray (see Exercise 1).
(2) Solution of Wilson and Wiseman[9] containing 0.04 μCi ^{45}Ca and 0.02 μCi ^{85}Sr/ml.
(3) Solution of Wilson and Wiseman containing 0.04 μCi ^{45}Ca/ml, 0.02 μCi ^{85}Sr/ml, and 10^{-3} M NaCN.
(4) Solution containing 0.04 μCi per ml ^{45}Ca.
(5) Solution containing 0.02 μCi per ml ^{85}Sr.
(6) Rats (preferably maintained on a low calcium diet for at least a week).
(7) Well-type scintillation counter with single-channel analyser and suitable scaler.
(8) G-M counter.
(9) Constant-temperature water-bath shaker set at 37°C.
(10) Oxygen tank with attached rubber hose.
(11) Sodium pentobarbital (65 mg/ml).
(12) 5-ml syringe and 20-gauge 2.5-cm needle.
(13) 1-ml tuberculin syringe and 20-gauge 2.5-cm needle.
(14) 5.0-ml volumetric pipette and control.
(15) 0.2-ml calibrated pipette.

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[9] Wilson and Wiseman solution:

NaCl	0.135 M
KCL	0.011 M
$CaCl_2$	4×10^{-5} M
Na_2HPO_4(pH 7.4)	0.008 M

(16) 15-ml graduated centrifuge tubes (2).
(17) 25-ml Erlenmeyer flasks with rubber stoppers (2).
(18) Counting tubes (8).
(19) Planchets (8).
(20) Scissors, scalpel, forceps, flexible probe.
(21) Cotton thread.
(22) Squeeze bottle of physiological saline solution.
(23) Infra-red heat lamp.
(24) Planchet trays.

Procedure

1. (1) Kill rat by injecting intracardially an overdose of sodium pentobarbital.
2. (2) Immediately incise the abdomen, resect the duodenum at the pylorus, and remove a segment of duodenum that measures about 8 cm. Do not pull mesentery free but cut mesentery with surgical scissors.
3. (3) Evert duodenal segment by inserting a flexible probe completely through the lumen, tying off one end and telescoping the gut segment through itself beginning at the tie (see Fig. 33).
4. (4) Wash the everted segment with saline, and tie at one end with surgical thread.
5. (5) Inject 0.6 ml of labelled incubating solution into other end of segment; inject into the segment through a second tie to prevent backflow. After injection, remove needle while pulling the second tie tight. Thus, a "gut sac" is formed with the serosa inside and the mucosa outside.
6. (6) Place sac in 25-ml Erlenmeyer flask containing 5 ml of incubating solution, and gas for 1 min with oxygen.
7. (7) Close flask with rubber stopper and incubate in water-bath shaker for 1 h.
8. (8) At the end of 1 h remove segment from flask, rinse with saline, and blot on absorbent tissue paper. Allow fluid inside of sac to run into a graduated 15-ml centrifuge tube and determine residual volume.
9. (9) Pipette 0.2-ml samples in duplicate from outside and inside solutions into stainless-steel planchets and dry under infra-red heat lamp. Count dried samples for ^{45}Ca beta activity.
10. (10) Pipette 0.2-ml samples in duplicate from outside and inside solutions into counting tubes and determine ^{85}Sr activity with a scintillation detector.
11. (11) Repeat above procedure with another rat but use Wilson-Wiseman solution containing 10^{-3} M NaCN.

Calculations and reporting of results

(1) Calculate the S/M ratios for strontium and calcium in the two animals. The S/M ratio is defined as the ratio of cpm/ml on the serosal side (inside of sac) to cpm/ml on the mucosal side

(outside of sac). Transport of an ion against a concentration gradient from the mucosal side to the serosal side will be indicated by S/M >1.

	cpm/ml ^{85}Sr		Ratio S/M	cpm/ml ^{45}Ca		Ratio S/M
	Inside (S)	Outside (M)		Inside (S)	Outside (M)	
Rat No. 1						
Rat No. 2						

Questions

(1) Is the observation that an ion is moved against a concentration gradient sufficient evidence for active transport?
(2) What forces, other than active transport, may account for movement against a concentration gradient?
(3) What other processes could cause ion movement across biological membranes?
(4) From the data, what mechanisms would you propose for the intestinal transport of Ca and Sr.

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9. IN-VITRO TRANSPORT OF SODIUM ACROSS FROG BLADDER

Objectives

(1) To measure the rates of movement of sodium ions in both directions across a biological membrane.
(2) To measure the electropotential produced by a biological membrane.
(3) To determine the type of transport by which sodium moves across a frog bladder.

Introduction and theory

Many biological membranes have the capacity to transport sodium ions against an electropotential gradient. The bladder of the frog has been extensively studied in this connection since it develops an electropotential differ-

2

ence of 70 - 100 mV, actively transports sodium, and is convenient to work with. For interpretation of the data from this type of experiment, Ussing derived the classical rate equation which permits thermodynamic evaluation of the processes that occur. This treatment is used in the present exercise.

Generally, the bladder from a pithed frog is placed between the two chambers of a diffusion cell (see Fig.34), and the chambers are then filled with Ringer's solution. The solution in one chamber is labelled with ^{22}Na ($T_{\frac{1}{2}}$ = 2.6 yr) while the solution in the other chamber is labelled with ^{24}Na ($T_{\frac{1}{2}}$ = 15 h). The unidirectional rate of each radioisotope is determined and the electropotential difference across the membrane is simultaneously recorded with a sensitive potentiometer.

If only one sodium isotope is available, the following modifications may be employed. Two diffusion cells are used, each containing a piece of tissue from the same frog. In the first cell, only the solution in contact with the outside of the tissue is labelled, whereas, in the other cell, the labelled solution is placed in contact with the inside. Another procedure is to label the solution on one side and to determine the transport rate for a specific length of time. The cell is then emptied, refilled with the labelled solution in contact with the other side of the tissue, and the transport rate determined.

From the values obtained during the experiment, it can be determined whether active sodium transport is taking place on the basis of the following considerations. If only simple diffusion were occurring, the rate ratio should be given by the theoretical equation:

$$\text{Rate ratio} = \frac{\rho_1}{\rho_2} = \frac{c_2}{c_1} e^{zFE/RT} \qquad (1)$$

where

ρ_1 is the transport rate of Na^+ from compartment 1 to 2 (μmole/h).
ρ_2 is the transport rate of Na^+ from compartment 2 to 1 (μmole/h).
c_1 is the Na^+ concentration in compartment 1 at zero time.
c_2 is the Na^+ concentration in compartment 2 at zero time.
z is the valency of Na^+ = 1.
F is the Faraday constant = 96 500 coulombs per equivelent.
E is the potential difference between compartments ($E_1 - E_2$).
R is the gas constant = 1.987 cal °C^{-1} mole^{-1} = 8.31 J °C^{-1} mole^{-1}.
T is the absolute temperature = 273 + °C.

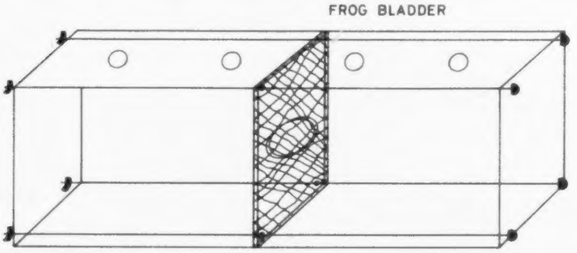

FIG. 34. Diffusion cell.

Under normal conditions, zF/RT is about equal to 1/25 mV. Since the initial concentrations of sodium ion on each side of the membrane are the same, Eq. (2) reduces to:

$$\frac{\rho_1}{\rho_2} = e^{\frac{E_1 - E_2}{25}} \qquad (2)$$

Thus it is necessary only to measure the potential difference in order to calculate the theoretical diffusion-rate ratio of Na^+. If the observed ρ_1/ρ_2 ratio as measured from the unidirectional transfer of radiosodium is equal to the theoretical rate ratio as calculated, i.e. (ρ_1/ρ_2) observed = (ρ_1/ρ_2) theoretical from Eq. (2), it is implied that Na traverses the membrane by simple diffusion. If the observed value of (ρ_1/ρ_2) is greater than the theoretical value of (ρ_1/ρ_2), active sodium transport is indicated. If (ρ_1/ρ_2) observed is less than (ρ_1/ρ_2) theoretical, sodium is thought to be transferred by a combination of exchange diffusion and simple diffusion. If (ρ_1/ρ_2) equals 1, the Na^+ is transferred by exchange diffusion.

By the use of constants from the literature, the current carried by the net rate of Na^+ can be compared with the total current crossing the membrane. The total current is calculated from the equation:

$$i = E \times C \qquad (3)$$

where

i is the total current in μA,
E is the potential difference $(E_1 - E_2)$ in mV,
C is the total conductance = 0.55 mmhos/cm^2.

Since the net transfer of 1 μ-equivalent per hour = 26.8 μA, the total μ-equivalents per hour can be estimated. These values for i can then be compared with the observed net Na rate $(\rho_1 - \rho_2)$. This will indicate what fraction of the current is carried by Na^+ ions.

An additional calculation can be made to estimate the work performed by the membrane in accomplishing the transport of sodium. Since there is no concentration difference, the only considerations are the work to overcome the electropotential difference, and to overcome the internal sodium resistance of the membrane. This is given by the equation:

$$W = zFE + RT \ln \frac{\rho_1}{\rho_2} \qquad (4)$$

where

W is the work in calories/μ-equivanent of Na^+ transported, and the other constants and variables are as defined above. By inserting appropriate values for the constants and rearranging, Eq. (4) becomes:

$$W = 23\,160\,(E + 0.058 \log \rho_1/\rho_2) \text{ calories}/\mu\text{-equivalent Na transported} \qquad (5)$$

If two isotopes of sodium are used, it is necessary to measure each accurately when both are present. A difficulty in the use of the gamma-ray spectrometer for this purpose is caused by the fact that the lower gamma-ray peak of ^{24}Na (2.758 MeV, 1.38 MeV) lies quite close to the higher gamma-ray peak of ^{22}Na (1.280 MeV, 0.510 MeV). This matter can be resolved in either of two ways.

In one procedure, the ^{24}Na is counted at its higher peak immediately after collection of the samples. The samples are saved for one week during which time the ^{24}Na ($T_{\frac{1}{2}}$ = 15 h) will have decayed to a negligible value. The samples are then counted again at the higher gamma peak of ^{22}Na ($T_{\frac{1}{2}}$ = 2.6 yr). The alternative method is to count a standard sample containing only ^{24}Na at the peak of ^{24}Na and at the peak of ^{22}Na. The amount of cross-counting at ^{22}Na peak can then be determined as a fraction of the count at the ^{24}Na peak. In like manner, a standard sample containing only ^{22}Na is counted at both peaks and the fraction of ^{22}Na counted at the ^{24}Na peak is determined. The samples may then be counted for both ^{22}Na and ^{24}Na after collection and the count of each isotope determined by the use of simultaneous equations.

Materials

(1) Standard tray.
(2) ^{24}Na and/or ^{22}Na solutions. Approximately 0.5 μCi of ^{22}Na should be used in each half cell while 1.0 μCi of ^{24}Na per half cell is required.
(3) Bull frogs.
(4) Well-type scintillation counters with single-channel analysers.
(5) Diffusion cells.
(6) Potentiometer (high impedance) measuring the 0-100 mV/range.
(7) Matched calomel electrodes.
(8) KCl-agar bridges (boil 28% KCl and 3% agar in H_2O, and draw into PE 200 polyethylene tubing with hypodermic syringe).
(9) Oxygen tank with suitable tubing.
(10) Ringer's solution.
(11) Petroleum jelly.
(12) 20-ml volumetric pipettes plus control (2).
(13) 1-ml volumetric pipettes (10).
(14) Counting tubes (10).
(15) Scissors, forceps, dissecting needle.

Procedure

(1) Grease all contact surfaces of the diffusion cell with petroleum jelly.
(2) Pith the frog and excise an area of bladder tissue measuring about 4 cm by 4 cm.
(3) Place the tissue between the two halves of the diffusion cell and clamp together. Some trimming may be necessary.
(4) To one chamber add 20 ml Ringer's solution labelled with about 5 μCi of ^{22}Na. To the other chamber add 20 ml Ringer's solution labelled with about 10 μCi of ^{24}Na.
(5) Aerate by placing in each chamber a small polyethylene tube attached to an O_2 tank. The bubbling will also serve to mix the solutions.
(6) Place one end of an agar bridge into each chamber and insert the other end into the calomel electrodes which are connected to the potentiometer. Keep the length of the bridges as short as possible and make sure there are good contacts at all points.
(7) Sample each chamber at 10, 20, 30 and 40 min, taking 1 ml at each sampling.
(8) Record continuously the electropotential generated by the membrane and note the potential at the time of each sample.

(9) Determine the ^{22}Na and ^{24}Na in each sample by counting at the correct peak with a well-type scintillation counter and a single-channel analyser or use the differential decay method.

Calculations and reporting of results

(1) Calculate the unidirectional rates of sodium.
(2) Calculate the theoretical rate ratio from Eq. (2).
(3) Calculate the total current and compare with the current due to Na^+ movement.
(4) Determine the amount of work performed by the membrane, assuming that active Na^+ transport does take place.

Questions

(1) From the data, what type of transport process is indicated for Na^+?

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10. CHOLESTEROL METABOLISM IN THE RAT AS STUDIED BY LIQUID SCINTILLATION COUNTING OF TRITIUM AND CARBON-14

Objectives

(1) To demonstrate a technique of sample preparation for liquid scintillation counting.
(2) To study the absorption and biosynthesis of cholesterol in the rat.

Introduction and theory

The basic principles of liquid scintillation counting have been treated in the Basic Part, Lecture Matter (Section 2.3.2) and in Laboratory Exercises (Section 2.4). In this exercise applications are made to the study of certain aspects of cholesterol metabolism in the rat.

Dietary cholesterol is readily absorbed from the intestinal tract of higher animals. The route of absorption appears to be via the lymphatics. Cholesterol alone is absorbed moderately well from the intestine of the rat, but many dietary liquids and fatty acids such as oleic and linoleic acids enhance the absorption of the sterol. A commonly held view is that fatty acids aid in the transport of cholesterol through the cell, or the exit from the cell, by providing an essential substrate for its esterification.

Although cholesterol is readily absorbed from the gut, it is not an essential component of the diet. The fact that most animals can synthesize their own sterols from smaller carbon units has been known for a long time, and the use of radioisotopic methods has provided important information on the metabolic pathways in sterol synthesis. In 1954 it was shown that acetate is an effective precursor of cholesterol and that both carbon atoms of the acetate radical are incorporated into the cholesterol molecule.

In the isolation of radioactive cholesterol from body tissues and fluids, care must be taken that the product is not contaminated with various precursors. Recently, a technique has been proposed for the precipitation of cholesterol using the glycosidal alkaloid tomatine, which is isolated from the stems and leaves of tomato plants. This technique appears to be more specific for cholesterol than any of the previous methods.

The following experiment is designed to demonstrate both the intestinal absorption of cholesterol-7-^{3}H and the biosynthesis of cholesterol from sodium acetate-1- ^{14}C.

Materials

(1) Standard tray (see Exercise 1).
(2) Cholesterol-7- ^{3}H.
(3) Sodium acetate-1- ^{14}C.
(4) Rats (100 - 200 g).
(5) Liquid-scintillation counter.
(6) Colorimeter.
(7) Water bath, steam bath, or sand bath.
(8) Centrifuge.
(9) Nitrogen-gas tank plus gauge.
(10) Petroleum ether, 60 - 70°C boiling range.
(11) Acetone-ethanol solution (1:1).
(12) Acetone, ethanol, ether, (4:4:1).
(13) Alcoholic KOH (50% wt/vol. in ethanol).
(14) Cholesterol solution: Dissolve 100 mg of cholesterol in 100 ml of glacial acetic acid. A working standard (0.02 mg/ml) is made by diluting 2 ml of stock solution to 100 ml with glacial acetic acid.
(15) Ferric chloride solution: Dissolve 1 g of ferric chloride hexahydrate in 10 ml of glacial acetic acid.
(16) Colour reagent: Pipette 1.0 ml of ferric chloride solution into a 100-ml volumetric flask and dilute to the mark with concentrated sulphuric acid.
(17) Glacial acetic acid.
(18) Tomatine solution:
    (a) 1% tomatine in ethanol   55 parts
    (b) water   44 parts
    (c) glacial acetic acid   1 part
(19) Scintillation solution:
    (a) PPO (2-5 diphenyloxazole)   4 g
    (b) POPOP (1-4-bis-2-5-phenyloxazolyl benzene)   150 mg
    (c) Toluene   make up to 1 litre
(20) Vegetable oil.
(21) Heparin solution (1000 I.U./ml).
(22) Ether.
(23) 15-ml centrifuge tube (1).
(24) 50-ml centrifuge tubes (2).
(25) Colorimeter tubes.
(26) 1-ml syringes with 24-gauge needles (2).

(27) 10-ml syringe with 20-gauge 2.5-cm needle.
(28) Stirring rods.
(29) Disposable pipettes.
(30) Assorted volumetric pipettes plus control.
(31) Ether chamber.
(32) Liquid-scintillation counting vials.
(33) Polyethylene stomach tube.

Procedure

(1) Administer to a rat by stomach tube 0.4 mCi/kg body weight of cholesterol-7-^{3}H dissolved in approximately 0.5 ml of vegetable oil.
(2) Leave the animal in a cage for 3 h after feeding.
(3) At the end of 3 h inject intraperitoneally into the rat 0.2 mCi/kg body weight of sodium acetate-1-^{14}C.
(4) Return the animal to the cage for an additional 1-h period.
(5) At the end of 1 h anaesthetize the rat and remove approximately 5 ml of blood in a heparinized syringe by heart puncture or from the vena cava after opening the abdomen.
(6) Centrifuge the blood sample for 15 min at 2000 rev./min.
(7) Pipette two 0.5-ml aliquots of plasma into 50-ml centrifuge tubes and add 0.5 ml of 50% alcoholic KOH.
(8) Heat on the water bath at 50 - 55°C for 1 h.
(9) Cool to room temperature, add 5 ml of petroleum ether and shake. Now add 5 ml of water and shake vigorously for 1 min. After two clear layers have formed, draw out the top layer. Repeat with another 5-ml volume of petroleum ether.
(10) Evaporate the solvent under nitrogen.
(11) Add 2 ml of acetone-alcohol (1:1) and then add 0.5 ml of the tomatine solution (solution 18).
(12) Incubate at room temperature for 20 min. At the end of this time a tomatinide precipitate should have formed.
(13) Centrifuge for 5 min to settle the precipitate and draw off the supernatant. Wash the precipitate with the acetone, ethanol, ether mixture, re-centrifuge and draw off the supernatant.
(14) Dissolve the precipitate in 5 ml of glacial acetic acid, heating gently if necessary.
(15) Pipette two 0.5-ml aliquots of the tomatinide solution into colorimetric tubes for total cholesterol determination. Add 2.5 ml of glacial acetic acid to each tube to make a total volume of 3.0 ml.
(16) Prepare two blanks containing 3.0 ml cholesterol and two standards containing 3.0 ml of the cholesterol standard (0.02 mg/ml).
(17) Arrange the tubes in a test-tube rack with a blank and standard at the beginning and the end of the series.
(18) Add 2.0 ml of colour-developing reagent (solution 16) to all tubes.
(19) Mix thoroughly using a very slowly flowing N_2 stream through a disposable pipette. If N_2 is not available, air may be used. Use a different pipette for each tube.
(20) Read the per cent transmission at 550 nm on the colorimeter provided.
(21) Place two 0.5-ml aliquots of the tomatinide solution into liquid-scintillation counting vials and add 20 ml of scintillation solution, mixing thoroughly.

(22) Prepare a blank containing 0.5 ml glacial acetic acid and 20 ml of scintillation fluid.

(23) Count the samples in the liquid scintillation counter at the optimum high voltage settings for ^{14}C and ^{3}H determined previously in the Basic Part (Section 2.4).

Calculations and reporting of results

(1) Calculate the cholesterol content in mg per 100 ml of plasma.

(2) Determine the specific activity (cpm/mg) of cholesterol-^{3}H and cholesterol-^{14}C.

Questions

(1) From the data what would you expect to be the best method of rapidly inducing a high cholesterol level in an animal?

(2) What sources of error might be present in the experimental technique?

(3) Since the tomatinide precipitation is not entirely specific for cholesterol, how could you determine the presence or absence of considerable amounts of contaminants (cholesterol precursors)?

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11. ASSAY OF TRITIUM IN MAMMALIAN TISSUES

Objectives

(1) To prepare animal tissues containing metabolically incorporated tritium.

(2) To demonstrate two methods for production of tritiated H_2O (for liquid scintillation counting) from animal tissues labelled with tritium.

(3) To demonstrate a method for the production of hydrogen gas and methane (for counting in an ionization chamber) from animal tissues labelled with tritium.

Introduction and theory

Because of the very low energy of the beta particles (0.018 MeV) from tritium (^{3}H) and consequent self-absorption, direct counting of tritiated substances using a gas-flow counter is not well suited for sensitive measurements. Usually, tritium is measured either by converting all the hydrogen of the sample to gaseous compounds which can be assayed in an ionization chamber or proportional counter, or by incorporation of the tritium into a liquid system suitable for scintillation counting.

The simplest method of preparing samples for liquid scintillation counting is to dissolve the activated substance directly in the scintillation fluid as is done in Exercise 11. However, this method often presents difficulties because of the light-absorbing properties of many biological materials. The colour, which may appear in the scintillation fluid when the sample is added, absorbs part of the light from the scintillations, thus resulting in a "quenching" effect. This quenching causes a lowered counting efficiency of the procedure. Chemical reactions between the sample and the phosphor may also result in quenching. While the use of internal standards or suitable correction curves may be employed to overcome this difficulty, it is often more expeditious to convert these samples to compounds which do not cause quenching.

One of the simplest methods of conversion is the combustion of the sample to produce water (see Procedure B.I.) which can be assayed conveniently and accurately by liquid scintillation counting. In the first experimental procedure, dried blood or tissue samples are converted into water, carbon dioxide and nitrogen by heating with copper and copper oxide at 650°C in sealed tubes with break-off tips. The tubes are broken in an evacuated apparatus and the tritiated water allowed to distill into a cold trap containing some ordinary water which acts as a scavenger for the small amount of tritiated water. The water is then flushed with 96% ethanol into a vial containing the scintillation counting solution and the radioactivity determined.

The second procedure (B. II.) is also based on combustion and is carried out in a thick-walled 2-litre flask (Schöniger flask) with a suitable ignition head fitted to a rubber stopper. The dried sample in a small bag made of visking membrane is ignited in an atmosphere of oxygen by means of an externally applied electric current. After combustion is complete, the bottom surface of the flask is placed in contact with a freezing mixture of dry-ice, chloroform and carbon tetrachloride. The water vapour within the flask then freezes on the bottom and 20 ml of scintillation solution containing 20% ethanol is added and swirled around the bottom of the flask to dissolve the ice. The flask is then placed in ice water for 10-15 min to attain a constant temperature and a 15-ml sample is placed in a scintillation vial for counting.

Conversion of the tritium in biological materials into a gas is the basis of the third procedure (C). The tritium is converted into methane and hydrogen by heating with zinc, nickelic oxide and water in a sealed tube at 640°C. This mixture may be used for ion-current measurements in the vibrating-reed electrometer and is also satisfactory for use in internal gas counters.

Materials

A. (1) Tritiated thymidine (10 μCi/rat).
(2) Rats (100-150 g).
(3) Cages.
(4) Heparin (1000 I.U./ml).
(5) Petri dish.

(6) 15-ml centrifuge tube.
(7) 5-ml syringe and 20-gauge needle.
(8) 1-ml tuberculin syringe and 20-gauge needle for injection.
(9) Scalpel, scissors, forceps.

B.I. (1) Tritiated biological samples.
(2) Balance accurate to 1.0 mg.
(3) High-vacuum pump.
(4) Constant-temperature water bath.
(5) Muffle furnace.
(6) Hand torch.
(7) Liquid scintillation counter
(8) Petroleum ether (for cleaning stop cocks and glass joints).
(9) Scintillation fluid

(a)	PPO	7.5	g
(b)	POPOP	75	mg
(c)	Naphthalene	120	g
(d)	Xylene	500	ml
(e)	Dioxane	500	ml

(10) 96% ethanol.
(11) High-vacuum stop-cock grease.
(12) Liquid nitrogen.
(13) 40-60 mesh copper oxide.
(14) 40-60 mesh reduced copper.
(15) Vacuum manifold.[10]
(16) Pyrex sample cups.[10]
(17) U-tube cold traps.[10]
(18) Drying tubes.[10]
(19) Combustion tubes.[10]
(20) Glass joints.[10]
(21) Distillation tubes with tops.[10]
(22) U-tube caps.[10]
(23) Accessory manifold glassware.[10]
(24) McLeod gauge (tilting).[10]
(25) 5-ml calibrated pipette with control.
(26) 10-ml volumetric pipette.
(27) Scintillation vials.
(28) Dewar flasks.
(29) Rubber tubing.
(30) Rubber tubing (high-vacuum).
(31) Bunsen burner.

B.II. (1) Tritiated biological samples.
(2) Liquid scintillation counter.
(3) Laboratory transformer or induction coil.
(4) Oxygen tank with attached rubber hose.

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[10] Obtainable from Delmar Scientific Glass Co., 4701 West Grand Avenue, Chicago, Ill., USA.

(5) Ignition head assembly (may be purchased or can be made with the following items):
  (a) Two-hole rubber stopper to fit 2-litre thick-walled flask;
  (b) Platinum wire 23 g;
  (c) Pyrex tubing;
  (d) Hand torch.
(6) Balance accurate to 1.0 mg.
(7) Dry ice, chloroform, carbon tetrachloride bath.
(8) Ice-water bath.
(9) Scintillation solution (as in Section B.I.) containing 20% ethanol.
(10) 20-ml volumetric pipette plus control.
(11) 15-ml volumetric pipette.
(12) Scintillation counting vials.
(13) 2-litre heavy-walled flask.
(14) Clamp to hold ignition assembly on flask.
(15) 2-cm-diam. seamless visking membrane.
(16) Haemostat, scissors.
(17) Adhesive tape.
(18) Cellophane tape or cellulose cement.
(19) Infra-red heat.

C. (1) Tritiated biological samples.
(2) Vibrating-reed electrometer.
(3) High-vacuum pump.
(4) Borkowski-type ionization chambers.
(5) Balance accurate to 1.0 mg.
(6) Muffle furnace.
(7) Hand torch.
(8) Nickelic oxide.
(9) Zinc.
(10) Methane gas.
(11) Pyrex reaction tubes.[11]
(12) Vacuum manifold.[11]
(13) McLeod gauge.[11]
(14) Gas-collection apparatus.[11]
(15) Pyrex tubing (for liquid samples).
(16) Small porcelain boats.

Procedure

A. Preparation of tritiated biological material

(1) Inject into a rat, intraperitoneally, approximately 10 μCi of tritiated thymidine. Place the animal in a cage for 1 h.
(2) At the end of 1 h, exsanguinate the animal either by heart puncture or decapitation, and collect the blood in a heparinized centrifuge tube; also remove the liver.

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[11] Delmar Scientific Glass Co., 4701 West Grand Avenue, Chicago, Ill., USA.

B.I. Combustion to produce water by Cu, CuO method

(1) Cut the fresh tissue into small pieces and place an amount representing 5 - 25 mg dry weight in a tared Pyrex cup.

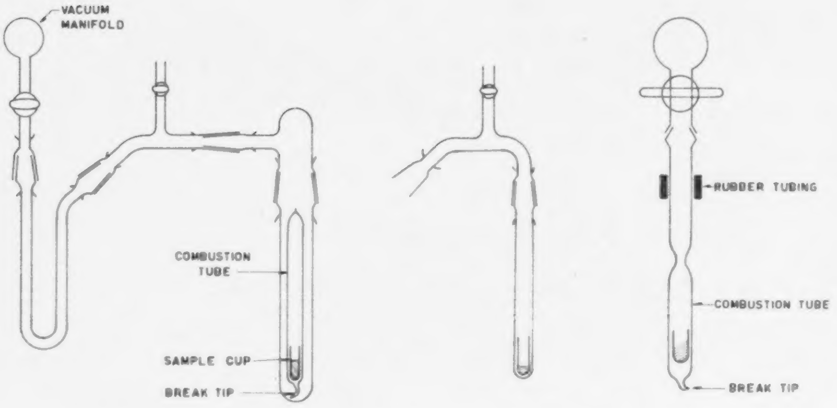
(2) Dry the sample by evacuation with an efficient oil pump for 30 min while the sample is immersed in a water bath at about 70°C (see Fig. 35(b)). For drying, tube sealing and water distillation, it is convenient to operate with a vacuum manifold equipped with a tilting McLeod gauge and having six ports about 15 cm apart so that six samples can be processed simultaneously.

(3) While drying condense the volatile constituents in a cold trap (liquid N_2) so that the radioactivity of this water can be determined.

(4) Weigh the cup with the dried sample and determine water of dehydration by difference. Add 0.75 g of 40-60 mesh copper oxide and 0.25 g of 40-60 mesh reduced copper.
(Copper oxide is prepared by grinding reagent-grade copper oxide in a mortar and pestle, or preferably in a ball mill, and taking the portion which passes a 40-mesh but not a 60-mesh sieve. The copper is obtained by passing a stream of hydrogen through the 40-60 mesh copper oxide in a tube heated to 300-350°C.)
Note: When liquid samples are being assayed (blood or tissue homogenates) the copper-copper oxide mixture is placed in the cup first and 0.3 ml of the liquid is added to it with subsequent drying from the frozen state.

(5) Place each cup in a combustion tube made of 11 mm outer diameter (o.d.) Pyrex # 1720 glass, one end of which has been drawn out to form a break tip (see Fig. 35(c)).

(6) Constrict the combustion tube about 14 cm from the break-tip end with a hand torch.



(a) DISTILLATION (b) DRYING (c) SEALING

FIG. 35. Apparatus for tritium assay.

(7) Connect the combustion tubes glass to glass inside a small piece of rubber tubing to the glass joints fitting into the manifold ports (see Fig. 35(c)).
(8) Evacuate the tubes to 1 mmHg with the oil pump and seal off at the constriction with a hand torch.
(9) Heat the sealed tubes in a horizontal position for 1 h in a furnace at 650 ± 10°C.
(10) Cool the tubes, agitate them to break up any lumps of uncombusted carbonaceous material and reheat for a second hour at 650°C.
(11) After cooling, connect the tubes to the distillation apparatus which contains 0.10 ml of water in the U-trap (see Fig. 35(a)).
(12) Immerse the trap in a cold bath and evacuate the system.
(13) Close the stop cock to the manifold, rotate the vessel holding the combustion tube (break-tip down) upwards around the horizontal glass joint, and swing down sharply to break the tip of the combustion tube.
(14) Re-open the stop cock momentarily to re-establish vacuum.
(15) Distil the water into the U-trap with the system shut off from the vacuum for 5 min.
(16) Follow this by a 10-min distillation period with the pump connection open during which time the vessel containing the combustion tube is heated with a Bunsen burner for 3 min.
(17) Remove the cold bath, admit air to the system, remove the U-trap and cap it quickly to prevent evaporation of tritiated water.
(18) Transfer the contents of the U-trap to a scintillation vial with 5.0 ml of 96% ethanol in two portions.
(19) Add 10 ml of scintillation fluid and determine the radioactivity in a scintillation counter.

B.II. Combustion to produce water by Schöniger method

(1) Preparation of sample holder
    (a) Cut a 5-cm long, 2-cm-diam. seamless visking membrane.
    (b) Prepare a bag by folding one of the ends and sealing with a drop of cement or transparent tape.
    (c) Open the bag with a haemostat wrapped at the ends with adhesive tape, gum side up.
    (d) Cut the open bag into the shape of a ladle with a 2-cm "cup" as a sample holder. The "handle" will serve as a fuse for ignition and a tab by which to hang a wet sample for drying.
(2) Preparation and combustion of sample
    (a) Place about 10 mg of liver or 2 ml of blood in the sample holder and dry under infra-red heat lamp.
    (b) Place the dry sample in the platinum basket of the ignition head (see Fig. 36) and fold the fuse over the ignition filament.
    (c) Fill the heavy-walled flask with O_2 and immediately stopper tightly with the ignition-head assembly containing the sample, using a clamp to hold ignition head in place.
    (d) Using a laboratory transformer or an induction coil, allow current to flow through the ignition head until the combustion of the sample becomes self-sustained.

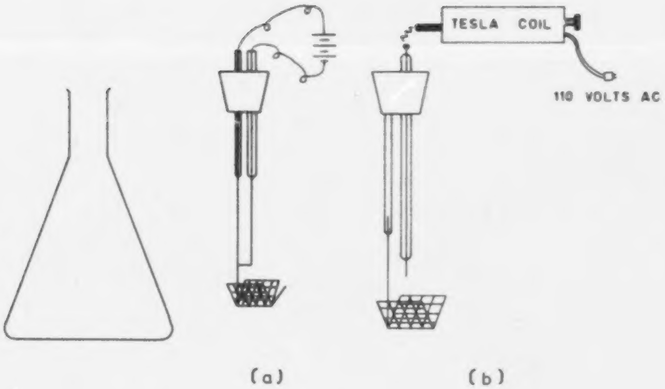


FIG. 36. Modified Schöniger flask and ignition heads.

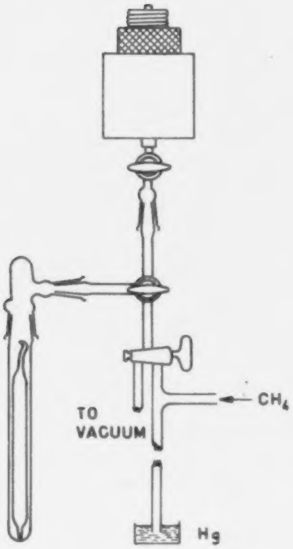


FIG. 37. Apparatus for analysis of gas samples.

(e) When combustion is completed place the bottom of the flask in a dry ice, chloroform, carbon tetrachloride bath for 30 min.
(f) At the end of the 30 min add to the flask 20 ml of scintillation solution containing 20% ethanol and swirl to dissolve the ice.
(g) Place the flask in ice water for 10 - 15 min to attain a constant temperature.

(3) Radioassay
(a) Pipette 15 ml of the solution in the flask into a scintillation counting vial.
(b) Count the samples in a liquid scintillation counter.

C. Preparation of hydrogen gas and methane (zinc fusion)

(1) Prepare reaction tubes 17 cm long with a break tip from 11 mm Pyrex # 1720 glass tubing.
(2) In each tube place 1.0 g of zinc, 100 mg of nickelic oxide and 5-6 mg of water.
(3) Add a 5.0 - 10.0 mg sample of the tritium-labelled tissue. Introduce liquid samples in a small sealed Pyrex ampoule with a break tip, and solid samples in a small porcelain boat.
(4) Constrict the reaction tube and evacuate to a pressure of 1 mm on the manifold.
(5) Seal off the reaction tube with a hand torch and agitate until the ampoule is broken and its contents evenly distributed throughout the reaction mixture.
(6) Place the reaction tubes in a furnace and heat at 640 ± 10°C for 3 h.
(7) At the end of the 3 h, allow the reaction tubes to cool.
(8) Place the reaction tube in the gas collection apparatus (see Fig. 37).
(9) Evacuate the system, close off the vacuum line, and invert the sample chamber to break the tip of the reaction tube thus allowing the gas to expand into the evacuated Borkowski-type ionization chamber.
(10) The fraction of gas entering the chamber is calculated from the known volume of the gas-collection apparatus plus ionization chamber corrected for the gas displacement by the reaction tube.
(11) Fill the chamber to atmospheric pressure by addition of methane.
(12) Measure the ion current collected at 360 V with a vibrating-reed electrometer.

Calculations

(1) Determine the concentration (μCi/mg) of tritium in the tissues by the various techniques used.

Questions

(1) Of the methods used, which in your opinion is the most accurate for the determination of tritium?
(2) What are the advantages and disadvantages of these techniques?
(3) Could any of these techniques be modified and used for the determination of ^{14}C? If so, what modifications are necessary?

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12. AUTORADIOGRAPHY

Objective

To illustrate the use of various autoradiographic procedures with tissue of animal origin.

Introduction and theory

The general concepts of autoradiography have been covered in the Basic Part of this book. Autoradiography provides visualization of the location of radionuclides within a sample. Applications range from those on a microscopic level which permit study of individual cellular or subcellular elements to those on a gross scale which, for example, are useful for the evaluation of macrodeposition in tissues and paper chromatograms. Autoradiography often can provide information that is unobtainable by counting methods. This is especially so for heterogeneous samples that contain regions of interest which cannot be separated mechanically. Perhaps of greatest import from the biological standpoint, however, is that autoradiography permits the study of cellular function at the cell or subcellular level since observations can be made of individual cells or subcellular structures from among millions. Thus, it becomes possible to develop correlations between the location pattern of specific chemical elements introduced into the system and cytological structure, cellular physiology and pathology, and physicochemical properties of cells.

There are four commonly used procedures that differ primarily in the method of contact between the tissue section and emulsion. The first method, simple apposition (A), is suitable for macro-autoradiography only; the others (B.I.–B.III) can be used for micro-autoradiography as well. The methods are described briefly as follows.

A. Macro-autoradiography, simple apposition method

The specimen to be studied is placed in contact with the photographic emulsion and kept in contact. At the end of the exposure period the specimen is removed and the film developed. This method is rapid and simple. However, it does not allow good resolution because of poor contact. Therefore, although the method is quite satisfactory for gross or macro-autoradiograms, it is not adequate for cellular localization. This procedure has been widely used in studies with bones, frozen tissue, and paper chromatograms. Thin sections are not required but it is advisable to have a smooth surface for good apposition to the film. Reasonably smooth surfaces can be obtained by the use of an ordinary band saw with bones or frozen animal carcasses. Also, samples such as bones and teeth can be polished with an emery wheel. The tissue must be placed very close to the film but direct contact should be avoided because of the danger of chemical fogging. Usually a thin sheet of plastic film is placed between the tissue and the photographic film to avoid contact, and moderate pressure is maintained between the section and the film to ensure good apposition. For this purpose, a press of plywood or plastic with a sponge rubber liner has been found convenient. It is necessary to avoid excessive pressure since this may produce artifacts.

B. Micro-autoradiography

B. I. Mounting method

In this method the sections are mounted on the emulsion and remain permanently bound to it throughout the subsequent photographic and staining processes. The method is simple, and the contact, registry, and resolution are good, thus permitting studies on the cellular level. The autoradiogram and section are always matched and are observed simultaneously which allows correlation between structure and photographic image. Care has to be taken that there is no loss of radionuclide during the fixation and the processing of the tissue. This method has been widely used for localization of ^{131}I in the thyroid tissue and for studying blood smears and bone-marrow smears that have been prepared by applying such samples directly to the surface of the emulsion.

B. II. Stripping film method

In this method an emulsion is stripped from its base and flattened over the histological section or smear on a glass slide. The specimen can be stained either before contact with the film or through the film base after exposure. Unstained sections can be studied by phase microscopy. This procedure also offers the advantages of even emulsion thickness, good contact, constant registry, and excellent resolution. It permits good correlation of radioactivity with histological structure and has been widely used for detailed cytological studies at the cellular level.

B. III. Coating method

In this method the section is covered with a fluid emulsion which is allowed to harden and forms a permanent bond for subsequent exposure and processing. This procedure provides good contact and constant registry which leads to good resolution and permits satisfactory correlation of radioactivity with histological structure. The technique has proven quite satisfactory for detailed cytological studies with various animal tissues and is becoming the procedure of choice in many laboratories.

Resolution

Resolution is perhaps the most important consideration in the preparation of high-quality autoradiograms at the cellular or subcellular level. If there are two point sources of radiation in a sample, each will produce a small circle of darkening in the emulsion. If these areas overlap, it

TABLE VIII. CHARACTERISTICS OF SOME COMMERCIAL EMULSIONS RECOMMENDED FOR AUTORADIOGRAPHY

Type	Sensitivity	Resolution	Background	Recommendation
No-screen X-ray	High	Poor	High	Gross localization or minimal concentration
NTB	Medium	Very good	Low	Mounted histological sections
NTB^{3}	High for β-tracks	Very good	Increases rapidly	When β-tracks are to be observed
Liquid emulsion	High	Very good	Increases rapidly	When β-tracks are to be observed
Stripping film	Low	Very good	Low	Cellular localization

will be impossible to distinguish them in the autoradiogram. The greater the resolution, the closer together are the areas that can be distinguished from each other. In general, the following conditions promote increased resolution: (a) Low-energy radiation; (b) Radiation of high specific ionization; (c) Thin specimens; (d) Thin emulsions; (e) Fine-grained emulsions; (f) Minimum of scattering from film backing and slide; (g) Close contact between the specimen and emulsion.

Photographic film

Various photographic emulsions have been especially developed to provide characteristics of advantage for particular autoradiographic application. Table VIII describes some of the commercial emulsions now available with recommendations for their use.

Radioactivity content and exposure times

In the production of a satisfactory autoradiogram there is an inverse relationship between the amount of radioactivity present in the tissue and the required time of exposure of the film. There is no set rule in regard to the amount of radioactivity that is required. A balance must be struck between large amounts of radioactivity that might produce radiation effects and small amounts that require unduly long exposure periods. In general, it has been stated that approximately five to ten billion beta particles per square centimetre of emulsion will be sufficient to cause adequate blackening. With alpha particles, one to two million particles per square centimetre are required. For guidance, it has been shown that with macro-autoradiograms, a section monitored with an end-window counter that gives a measurement of about 100 cpm at a counting yield of 10%, requires about a 10-15 d exposure period. In practice, the best way to determine the exposure times is to carry out preliminary investigations with graded levels of radioactivity. In this way the optimum exposure time can be determined.

Quantitation with autoradiograms

Autoradiography is used principally to obtain qualitative information on the distribution of radioactivity in biological material. However, it can also provide quantitative data as to the amount of radioactivity deposited in particular regions. This can be done by estimation of the photographic density of a given region, and calibration against a film exposed to known activities of the radioisotope in question under identical conditions. Comparative quantitative autoradiography can also be done by the counting of the grains produced per unit area. In this procedure it is necessary to have a low degree of darkening in the autoradiogram.

Sources of error in autoradiograms

Some of the major sources of error in autoradiographic procedures are:

(1) Removal or relocation of the radioactive atoms by biological or physical processes during the time between sampling and formation of the photographic image.
(2) Extraneous sources of image production, such as chemically active substances in the specimen; pressure on the emulsion; radioactivity in the films, chemicals, or glass used in processing; and stray light or ionizing radiation.
(3) Fading of the latent image or desensitization of the emulsion by the specimen.
(4) Non-uniform development.
(5) Scratches in the film; deposition of debris.
(6) Effects of staining solution on the emulsion.
(7) Movement of sample on film during exposure.

Many of these uncertainties have been eliminated in the procedures that are described in this exercise. It is always prudent, however, when making a series of autoradiograms, to include similar samples with no radioactivity as a control for errors that may tend to produce extraneous images.

Materials

A. (1) High specific activity $^{45}CaCl_2$ solution (50 μCi/ml).
(2) Young rats.
(3) Cages.
(4) Refrigerator.
(5) Darkroom facilities and safe-lights.
(6) Ether chamber.
(7) 2-ml syringe and 24-gauge needle.
(8) No-screen X-ray film.
(9) Plywood and sponge rubber press.
(10) Cardboard X-ray film holders (cassettes).
(11) Developing and fixing solutions.
(12) Scalpel, scissors forceps.
(13) Thin plastic sheeting.

B. I. (1) Celloidin-coated mounted sections of ^{131}I-activated thyroid gland.
(2) Refrigerator.
(3) Darkroom facilities and safe-lights.
(4) Developing and fixing solutions.
(5) Clean microscope slides.
(6) No-screen X-ray film.
(7) Scissors.
(8) Marking pencil.
(9) Aluminium foil.
(10) Labels.
(11) Tape.
(12) Small paper clamps.

B. II. (1) Thyroid-gland slides as in Part B. I.
(2) Refrigerator.
(3) Darkroom with Wratten No. 1 filtered safe-lights.
(4) Autoradiographic plates, AR-10, Kodak Ltd., England.
(5) Drying fan.
(6) Time clock.
(7) Methylene blue.
(8) Azure A.
(9) Glycerol C. P.
(10) Methanol C. P.
(11) KH_2PO_4.
(12) $Na_2HPO_4 \cdot 2H_2O$.
(13) $NaHSO_3$.
(14) Basic fuchsin (aqueous).
(15) Absolute ethanol.
(16) Xylene.
(17) Cedarwood oil.
(18) Anhydrous $CaSO_4$.
(19) Canada balsam.
(20) Developing and fixing solutions
    (a) Eastman Kodak D19 diluted 1:1 with distilled water is a suitable developing solution.
    (b) Eastman Kodak acid fixer with hardener is a good fixative.
(21) Large shallow dishes for floating emulsion.
(22) Slide-carrying racks with silver-plated wire handles.
(23) Cover slips.
(24) Staining dishes (20).
(25) Plastic slide boxes containing one clean microscope slide.
(26) Black friction tape.
(27) Wax marking pencil.
(28) Labels.
(29) Razor blades.

B. III. (1) Thyroid-gland slides as in Part B. I.
(2) Refrigerator.
(3) Darkroom facilities as for Part B. II.
(4) Constant-temperature water bath.
(5) NTB^3 liquid emulsion, Eastman Kodak, Rochester, N. Y., USA.
(6) Time clock.

(7) 50-ml beakers (2).
(8) Medicine droppers.
(9) Drying racks (for holding slides in a vertical position).
(10) Absorbent tissues.
(11) Spreaders (raclettes).
(12) Materials for developing, staining and mounting as in Part B. II.

Procedure

A. Macro-autoradiography

(1) Inject intraperitoneally into a young rat approximately 50 μCi of a high specific activity solution of $^{45}CaCl_2$ and place the animal in a cage for 1 h.
(2) At the end of 1 h kill the animal in an ether chamber.
(3) Remove the femurs and split them longitudinally with a sharp scalpel.
(4) Under safe-light conditions (darkroom) obtain a sheet of no-screen X-ray film (approximately 20 cm × 25 cm) and cover it with a sheet of very thin plastic film.
(5) Place the pieces of bone, cut side down, on the film and insert the film with applied bones in a cardboard film holder.
(6) Put the film holder in a plywood and sponge rubber press and tighten the screws.
(7) Place the press in a refrigerator at 4°C and expose for a period of 48 h.
(8) At the end of the exposure period develop the film according to the directions of the manufacturer.

B. Micro-autoradiography

Suitable mounted sections for these procedures may be prepared by obtaining the thyroid gland of a dog 24-48 h after an injection of 0.5 to 1.0 mCi of ^{131}I, inbedding it in paraffin, and cutting sections 5-10 μm thick. After preparation of the mounted slides, remove the paraffin by dipping for 2 min in each of two jars of xylene. Transfer to absolute ethanol (2 changes), dip in 1% celloidin for 30 sec, dry till tacky in vertical position and dip again in 1% celloidin. Dry the slides in a vertical position and store in a dust-proof container (plastic slide box).

B. I. Mounting technique (direct apposition)

(1) Cut 2 sheets of aluminium foil of appropriate size to wrap the specimens.
(2) Prepare a label to mark the package.
(3) Under safe-light conditions (Wratten No. 1) open a box containing pieces of no-screen X-ray film previously cut to the size of the microscope slides.
(4) Place the emulsion side of the film in contact with a celloidin-coated slide bearing a specimen of thyroid tissue containing ^{131}I, place a second clean microscope slide against the other side of the film (Fig. 38).

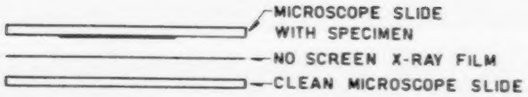

FIG. 38. Arrangement of a specimen and an X-ray film.

(5) Hold firmly and apply clamps on both sides.
(6) Wrap twice with aluminium foil fastening edges with tape.
(7) Place in a refrigerator at 4°C for 2-d exposure.
(8) At the end of the exposure time, develop the X-ray film according to the manufacturer's directions.

B. II. Stripping film technique

(1) Under safe-light conditions (Wratten No. 1) outline areas of the emulsion about 4 cm square with a razor blade. Let the plates stand for about 10 min before proceeding.
(2) Slip a razor blade under one corner of the outlined square and pull upwards slowly. (This will be difficult if the humidity is high.)
(3) Float the strip of emulsion face down on distilled water at 21-24°C, and let it soak for 2-3 min.
(4) Slip a mounted slide into the water beneath the floating film and lift the film out on top of the slide.
(5) Fold the free ends of the film underneath by turning the slide.
(6) Dry in front of a fan at room temperature for approximately $\frac{1}{2}$ h.
(7) When slides are dry place in a vertical position in a plastic slide box containing a small amount of anhydrous $CaSO_4$ trapped at one end behind a clean slide. Seal the edges of the box with black tape to ensure that it is light tight.
(8) Expose for 48 h in a refrigerator at 4°C.
(9) After 48 h, develop the slides according to the following procedure with the developer, wash bath, fixer, and wash sink all kept at 4°C.
  (a) Under safe-light, remove the autoradiographs from the exposure box and place them in a slide carrier all facing the same way. Attach a silver-plated holder to the carrier.
  (b) Immerse in developer for 10 min, agitating lightly once in a while.
  (c) Rinse by dipping slowly 15 times in a wash bath.
  (d) Fix for 15 min, then carry to wash sink for 15 min after which time the lights may be turned on.
(10) Staining the autoradiographs (Kingsley - basic fuchsin). Staining dishes and their contents are cooled to 4°C and maintained at this temperature in an ice water bath.
  (a) Stain the sections 5 min in a mixture of methylene blue and azure A according to Kingsley (1935):

Methylene blue		0.065 g
Azure A		0.010 g
Glycerol C. P.		5.0 ml
CH_3OH C. P.		5.0 ml
Distilled H_2O		25.0 ml
Phosphate buffer pH 6.9		15.0 ml
Composition of buffer:	KH_2PO_4	9.078 g
	$Na_2HPO_4 \cdot 2H_2O$	11.876 g
	H_2O	1.0 litre

- (b) Rinse in distilled water.
- (c) Destain 5-8 min in 0.05% aqueous $NaHSO_3$ until the emulsion is clear.
- (d) Wash 2 min in distilled water.
- (e) Counterstain 10-15 min in 0.05% aqueous basic fuchsin.
- (f) Remove excess stain in absolute ethanol.
- (g) Dehydrate 5 min in cold absolute ethanol.
- (h) Carry through two other ethanol baths, 5 min each, at room temperature.

(11) Clearing and mounting.
- (a) Transfer slides to a 1:1 mixture of absolute ethanol and cedarwood oil. Leave until the emulsion is entirely clear (5-15 min).
- (b) Drain off the excess and transfer to pure cedarwood oil (1.5-2 h).
- (c) Drain excess oil and place in a 1:1 mixture of xylene and Canada balsam (2-3 h).
- (d) Apply a cover slip with a liberal amount of Canada balsam dissolved in xylene.

B. III. Coating by the smear-drip method or by the dipping technique

For these procedures NTB^3 liquid emulsion, Eastman Kodak, Rochester, N. Y., USA, is required.

(1) Under safe-light conditions (Wratten No. 1) fill two 50-ml beakers with liquid emulsion and maintain in a water bath at 40°C.
(2) Take one thyroid-gland slide and dip it in the melted emulsion for 1-2 sec.
(3) Allow the excess emulsion to drain into the container.

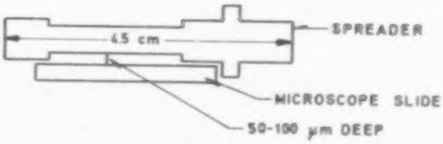

FIG. 39. Spreader for application of a liquid photographic emulsion.

(4) Wipe the back of the slide with an absorbent tissue.
(5) Place the slide on a drying rack for 30 min.
(6) If spreaders (raclettes) (see Fig. 39) are available, proceed as follows:
  (a) From the second beaker pick up some liquid emulsion with the medicine dropper.
  (b) Place four drops of emulsion at the inside end of one of the thyroid-gland slides.
  (c) Take the spreader and grasp firmly at both ends with the other hand.
  (d) Place it gently on the slide behind the emulsion layer.
  (e) Carry it outward slowly, thus coating the slide evenly with 100 μm of liquid emulsion.
  (f) Place the slide vertically on a folded piece of absorbent tissue and allow the excess emulsion to drain off (30-40 sec).
  (g) Place in a drying rack, emulsion face out. Leave for 30 min.
(7) Exposure, developing, staining, and mounting procedures are followed according to Section B. II.

Examination of autoradiographs

Examine with microscope stage in horizontal position.
(a) Look for definition of contact, coated, and strip-film preparations.
(b) Examine for background and size of grain.
(c) Examine for artifacts (chemical or mechanical fog, leaching, wrinkles, air-bubbles).
(d) Compare distribution and intensity of the autoradiographic record over different cells of the various sections.
(e) Compare quality of staining with different autoradiographic procedures and different emulsions.
(f) Prepare a report of your activities and observations.

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13. ACUTE EFFECTS OF RADIATION ON THE RAT

Objectives

(1) To observe irradiation-induced changes in the blood and other tissues of the rat.
(2) To follow the pattern of the acute radiation syndrome by comparison of unirradiated (control) rats with rats exposed to 700 R of X- or gamma rays.

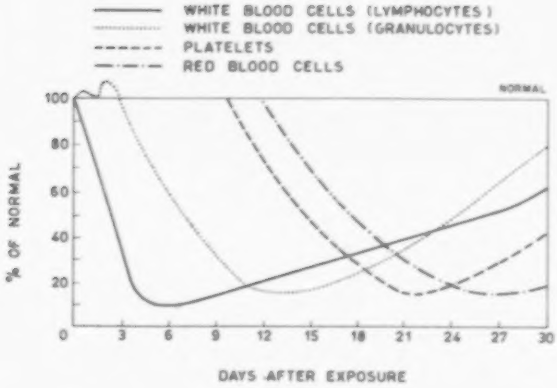

FIG.40. Effect of irradiation on formed blood elements.

Introduction and theory

It has been recognized since earliest times that ionizing radiations produce deleterious changes in living tissues. The term "acute radiation syndrome" has been given to the collective effects which are seen in mammals following doses of X- or gamma-radiation which are of the order of the $LD_{50/30}$ (the dose of radiation that will kill 50% of those individuals exposed within thirty days of exposure).

The acute radiation syndrome may manifest itself in three different ways depending upon the dose of radiation received. When the whole-body dose received is of the order of 200-800 R, the "haemorrhagic syndrome" occurs. This terminology is used because most of the pathologic effects are seen in the blood and blood-forming organs. In general, the pathogenesis is as follows. Within the first 24 to 48 h after exposure the lymphocytic cells drop markedly to 0-10% of their normal value. The granulocytes usually show a transitory rise in the number of circulating cells followed by a gradual drop to approximately 25% of their normal value in 1 to 2 weeks.

Platelets and red blood cells show more latent changes due to the longer life-span of these elements. A gradual drop in the number of circulating red blood cells begins about 2 weeks after exposure with the most severe anaemia occurring at 3 to 4 weeks post-exposure. If the animal recovers, the blood cells begin a slow rise approaching normal values in 6 months to 2 years. However, they may never regain the pre-irradiation levels. Figure 40 summarizes the effects on the formed elements of the blood.

Changes in the bone marrow can be seen within 1 hour after exposure and are manifested by a decrease in the number of erythroblasts. After 9 or 10 days, the marrow cavity is virtually devoid of cells and contains a gelatinous mass with a few fibroblasts, blood vessels, and reticular fibres as the only vital elements. If the animal survives, regeneration begins with the development of foci of primitive myelocytes and erythroblasts about the reticular elements; eventually, the marrow may be completely reconstituted.

Fatalities from the haemorrhagic syndrome usually occur about the 10th to the 14th day after exposure due to the profound bacteraemia that ensues when the granulocytes drop to their lowest values.

Radiation levels ranging from 1000 to 10 000 R whole-body dose lead to the "gastro-intestinal syndrome". This syndrome is characterized by nausea and vomiting beginning within a few hours of exposure. Vomition is followed by a haemorrhagic diarrhoea which leads to a severe dehydration. Death usually occurs in 3 to 4 days.

The explanation for this three- to four-day survival is that division of the epithelial cells of the gut is inhibited and as the cells die (in the course of about three days) no replacement occurs. The absorption of foodstuffs is impaired, dehydration occurs as a result of diarrhoea, the resistance to bacterial invasion from the gut is abolished, and a fatal bacteraemia ensues.

If the dose received is in excess of 10 000 R total body dose, death occurs within twenty-four hours and the "central nervous syndrome" is exhibited. The victim usually becomes comatose within 15 minutes of exposure. Brain damage may be a direct result of the radiation or secondary to vascular destruction.

In this exercise an attempt will be made to produce the haemorrhagic syndrome in a group of rats. However, bearing in mind the large species and individual variations, the gastro-intestinal syndrome may also be produced in some animals.

Materials

(1) 200 kVp X-ray machine or 50-100 Ci ^{60}Co source.	
(2) Mature rats.	
(3) R-chamber or other suitable dosimeter.	
(4) Rat scale.	
(5) Balance accurate to 1 mg.	
(6) Light microscope.	
(7) Microcapillary centrifuge.	
(8) EDTA.	
(9) Türk solution (for WBC)	
(a) Glacial acetic acid	7.5 ml
(b) Distilled H_2O	500.0 ml
(c) Gentian violet	1 crystal
(10) Hayem solution (for RBC)	
(a) NaCl	2.0 g
(b) Na_2SO_4	10.0 g
(c) $HgCl_2$	1.0 g
(d) Distilled H_2O	400.0 ml
(11) Wright's stain powder	
(a) Wright's stain	0.2-0.3 g
(b) Methyl alcohol, absolute	100 ml
(12) Immersion oil.	
(13) Xylol.	
(14) Ether chamber.	
(15) Disposable spot plates.	
(16) Microscope slides.	
(17) Glass rods (for use as droppers).	

(18) WBC pipettes.
(19) RBC pipettes.
(20) Haemocytometer (blood-cell counting chamber).
(21) Microhaematocrit (blood-cell counting chamber).
(22) Rubber tubing and mouthpieces (for blood pipettes).
(23) Micro-haematocrit reader.
(24) Dissection kit.
(25) Cotton sponges.
(26) Surgical scissors.
(27) Rat metabolism cages.

Procedure

A. Procuring blood: preparation and counting of blood samples

(1) Anaesthetize a rat in an ether chamber.
(2) When animal is under anaesthetic, draw blood as follows:
  (a) Swab the tail with a sponge moistened in xylol.
  (b) Immerse the tail in warm water for 30 sec and gently dry with a sponge or paper tissue.
  (c) Sever the distal (1-2 mm) tip of the tail with a single incisive cut from sharp scissors.
  (d) Discard the first two or three drops and then let 3 to 6 drops of blood fall into a disposable spot-test plate containing EDTA (anticoagulant) and stir gently.
(3) Place the animal back in its cage.
(4) Prepare blood slides as follows:
  (a) Place a small drop of blood about 2 cm from the end of a clean microscope slide.
  (b) Using a second slide as a spreader, hold the end of the second slide at an angle of 45° or more against the surface of the slide containing the blood and ahead of the blood drop.
  (c) Back the spreader into the blood and push it slowly and smoothly in the opposite direction across the surface of the slide producing a thin smear.
  (d) Label the slide and set it aside to dry.
(5) Fill a WBC pipette exactly to the 0.5 mark. Promptly place the tip of the pipette into the WBC dilution fluid (Türk solution) and slowly fill to the 11 mark.
(6) Holding the pipette by the ends between the thumb and middle finger, shake for about 1 min to disperse the cells and set the pipette aside horizontally.
(7) Repeat the procedure with an RBC pipette, using Hayem's solution as diluting fluid and filling to the 101 mark.
(8) Fill two micro-haematocrit capillary tubes about 3/4 full with tail blood and seal the empty end with the Bunsen burner.
(9) Spin down the samples and determine the haematocrit.
(10) Determine the total leucocyte count as follows:
  (a) Place the counting chamber (haemocytometer) in a horizontal position with the cover slip in place over the ruled areas.
  (b) Shake the pipette 2 min and discard 2 or 3 drops.

(c) Fill the chamber immediately by placing a finger over the upper end of the pipette and, while holding it almost horizontal, touch the tip to the ruled area at the edge of the cover slip. Slowly release the finger and allow the fluid to fill the space under the cover slip by capillarity. Do not overfill.

(d) Allow 1 min for the leucocytes to settle.

(e) Count four corner groups of sixteen squares under low power (see Fig. 41). Include in the count the cells falling on the upper or left sides of the square but omit those falling on the lower or right borders.

(f) The sum of the number of cells counted in the four squares, when multiplied by fifty, gives the total leucocyte count per mm^3.

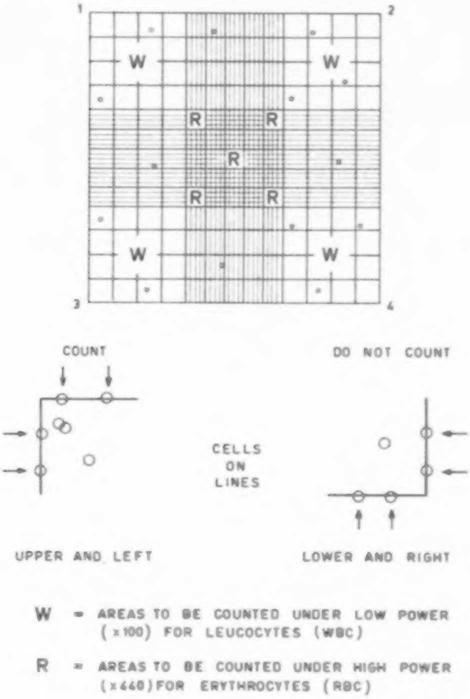
1
2
W
W
R
R
R
R
R
W
W
3
4
COUNT
DO NOT COUNT
CELLS ON LINES
UPPER AND LEFT
LOWER AND RIGHT
W = AREAS TO BE COUNTED UNDER LOW POWER (×100) FOR LEUCOCYTES (WBC)
R = AREAS TO BE COUNTED UNDER HIGH POWER (×440) FOR ERYTHROCYTES (RBC)

FIG. 41. Chamber for counting erythrocytes and white blood corpuscles.

(11) Determine the total erythrocyte count as follows:

(a) Fill the counting chamber in the same way as was done for the white cell count.

(b) Count only the four corner squares and one centre square in the centre block of 25 squares (Fig. 41). Use high power. The sum of the number of cells counted in the five squares, when multiplied by 10 000, gives the total erythrocyte count per mm^3.

(12) Stain the smears for differential count as follows:
  (a) Place the smears horizontally on screening or corks.
  (b) Drop Wright's stain on the slides until the entire surface is flooded and let stand for 3 min.
  (c) Add an equal amount of distilled water and mix by blowing very gently across the surface. Let the preparation stand for 9 min.
  (d) Wash off the stain by running a stream of H_2O directly into the centre of the slide.
  (e) Allow smears to air dry.

(13) Inspect the slides under high-power objective to locate the best area to count.

(14) Add a drop of immersion oil and switch to the oil-immersion objective.

(15) Count 100 leucocytes and report percentage of lymphocytes and percentage of granulocytes. Count 100 consecutive white cells following parallel horizontal tracks across the slide.

B. Irradiation studies

(1) Obtain blood values (haematocrit, RBC, WBC, differential) on four numbered rats.

(2) Determine the dose-rate of the X-ray machine or gamma-ray source with an R-chamber or any other suitable dosimeter.

(3) Irradiate two of the rats with a dose of 700 R total body of X- or gamma radiation.

(4) Confine all four animals in metabolism cages and keep daily records of body weight, water and feed consumption, and gross clinical observations such as diarrhoea, shaggy coat, irritability, etc.

(5) Obtain blood values every other day for a week and then twice a week for the next two weeks.

(6) Autopsy all animals dying during the course of the experiment.

(7) At the end of the 3-week period, sacrifice all remaining animals in an ether chamber and perform a complete autopsy noting any differences between the organs of irradiated and control animals, e.g. weight, haemorrhage, etc.

Reporting of results

(1) Prepare a graph of the blood changes in the irradiated rats over the experimental time period plotting % of normal values versus time as the abscissa. The mean blood values of the controls may serve as the normal values.

(2) List any differences noted between the organs of the irradiated and control animals and give your ideas as to the pathogenesis.

Questions

(1) Do you consider that the dose given to these animals exceeded the $LD_{50/30}$ for the rat?

(2) What tissues are most susceptible to radiation injury?

(3) What tissues are least susceptible?

(4) Can you formulate a hypothesis relating the rate of multiplication and degree of differentiation of a tissue to radiosensitivity?

(5) One school of thought feels that all tissues are equally sensitive to radiation damage. From your observations, either support or refute this theory.

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14. PREPARATION OF A PLASMA PROTEIN TRACE-LABELLED WITH RADIOIODINE

Objective

To demonstrate a simple technique for the in-vitro labelling of proteins with radioiodine.

Introduction and theory

Proteins containing radionuclides can be prepared by biological labelling, i.e. by the administration to an animal, microorganisms or cell culture of a suitably labelled amino acid which is then incorporated into protein during the normal synthetic processes. Proteins tagged in this way might appear to be the most desirable materials for many biological investigations, being completely native. The method has, however, two limitations: (a) because of the lack of specificity in the incorporation of the labelled amino acid, the particular protein required usually has to be separated from a mixture of similarly labelled materials; (b) the specific activity of the final product is likely to be low.

An alternative to biological labelling is to tag the protein in vitro by attaching a foreign radioactive atom. The most successful application of this method so far has been the preparation of proteins tagged with radioiodine. If suitable precautions are taken during the labelling with respect to the conditions and the amount of iodine introduced, tagged proteins of very high specific activity which are biologically indistinguishable from the natural protein, can be prepared by this method. It has been shown that the iodine label remains firmly attached to the protein under physiological conditions and is liberated only when the protein molecule is degraded.

The method described below for trace-labelling depends on treating the protein, in slightly alkaline solution, with iodine monochloride to which has been added the radioactive iodine as carrier-free iodide.

The preparation is one of tagged rabbit-serum albumin which is suitable for use in Exercise 16, but the method is general and can be appropriately modified for the production of other labelled plasma proteins in different amounts and with different specific activities.

Materials

(1) Carrier-free radioiodide (^{131}I or ^{125}I). This preparation must be free from reducing agents.
(2) Iodine monochloride: a solution containing 0.42 mg I/ml as ICl in molar NaCl and approximately 0.01N with respect to HCl.
(3) Buffers:
    Glycine buffer A – 9 ml molar glycine in 0.25M NaCl + 1 ml N NaOH
    Glycine buffer B – 8 ml molar glycine in 0.25M NaCl + 2 ml N NaOH.
(4) Gel-filtration equipment and sephadex (see Exercise 6) or dialysis tubing.
(5) An approximately 2% solution of rabbit serum albumin.

Procedure

(1) Place 4.0 ml of 2% rabbit serum albumin solution in a 100-ml conical flask and add 2.0 ml of glycine buffer B. Steps 2-4 are to be carried out in a ventilated fume hood.
(2) With a long-barrelled teat-pipette transfer approximately 1 mCi of carrier-free iodide solution to a tube containing 1.0 ml of the ICl solution and mix.
(3) With the same pipette add the now labelled ICl solution to a tube containing 2.0 ml of glycine buffer A, mix and immediately add the whole solution to the albumin preparation in the conical flask. The iodinating mixture should be jetted into the protein solution from the teat pipette and the flask swirled using a remote-control clamp at the neck.
(4) Allow a few minutes for the iodination to be complete and then add "carrier" protein, e.g. freeze-dried bovine serum albumin, to bring the specific activity to less than 5 μCi/mg. This reduces the risk of radiation decomposition.
(5) Remove unbound isotopes from the preparation either by gel filtration (Exercise 6) or by dialysis for at least 48 h against a large volume of physiological saline.
(6) After dialysis or gel filtration check the proportion of unbound activity in the preparation as follows: Place one drop in a 15-ml graduated conical centrifuge tube, add one or two drops of serum or similar protein solution to provide more carrier and make up to 5 ml with water. Add 5.0 ml of 20% trichloracetic acid, mix thoroughly and centrifuge. Remove the supernatant carefully with a teat pipette and retain it. Disperse the precipitate in a few drops of water, dissolve it by the addition of dilute NaOH and finally make up to 10 ml. By means of the well-type counter

compare the total radioactivity of the supernatant (unbound activity) with that of the dissolved precipitate solution (bound activity). For many experiments it is desirable that the unbound activity should be less than 2% of the bound activity.

Notes

A. Uses

The tagged rabbit-serum albumin prepared in this exercise can be used in various ways, e. g. in the measurement of plasma volume in rabbits (cf. Exercise 2); in the measurement of catabolic rate of serum albumin (Exercise 16); as a labelled antigen in immunological studies.

B. Quantitative aspects of the labelling

It is usual to aim at a trace-labelled preparation containing an average of 1/2 to 1 atom of iodine per molecule of protein; the amount of ICl solution used is adjusted accordingly. With the quantities described above, one would obtain the incorporation of about 2 atoms of iodine per molecule of albumin assuming 100% incorporation. In practice, albumin gives usually 30-40% incorporation of iodine under these conditions, so the level of iodination is likely to be acceptable. The amount of isotope added to the preparation can be varied to give preparations of any desired specific activity.

C. Potential health hazards

Since iodine isotopes will tend to concentrate in the thyroid, it is important that the precaution of carrying out certain stages of the preparation in a hood be strictly adhered to in case there is any ingestion or inhalation. Periodic checks on thyroid activity of the workers involved is recommended.

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15. MEASUREMENT OF THE CATABOLIC RATE OF PLASMA ALBUMIN IN RABBITS

Objectives

(1) To illustrate the use of trace-iodinated proteins in turnover studies.
(2) To compare the catabolic rate for plasma albumin in a normal rabbit with that in a fluke-infected one.

Introduction and theory

The amount of any plasma protein in the body at a given time represents a balance between synthesis and catabolism. When a reduction occurs in a body pool of protein, it is important to know whether this is due to a subnormal synthesis, an increased rate of catabolism or both. Proteins trace-labelled with radioiodine are particularly suited for the measurement of catabolic rates because there is no re-utilization of the label once the protein has been degraded. Provided the thyroid of the experimental animal is blocked by the administration of stable iodide, the label from degraded protein is excreted largely in the urine. In practice a sample of the labelled protein is injected intravenously and the plasma volume determined by applying the dilution principle. Blood samples are collected daily and complete 24-h collections of urine and fæces made over a period of 7-10 d. After counting suitable samples of urine, fæces and plasma the fractional catabolic rate (K), i.e. the fraction of the total intravascular pool degraded during 24 h, is calculated by dividing the total excreted radioactivity over that period by the total intravascular activity at the beginning of the collection period.

It is instructive to measure K in a normal rabbit and in a rabbit showing hypercatabolism of albumin, e.g. a rabbit infected with *Fasciola hepatica.*

Materials

(1) Rabbit serum albumin trace-labelled with radioiodine. The material prepared in Exercise 15 should be suitable.
(2) Rabbits: a normal rabbit and one infected at least eight weeks previously with *F. hepatica.*
(3) Metabolism cages for rabbits.
(4) A solution containing 0.005% NaI and 0.47% NaCl.
(5) Syringes, needles, etc. suitable for intravenous injection of rabbits.
(6) Micro-pipettes, e.g. "auto-zero" type, to measure volumes of 0.20 ml.

Procedure

(1) Establish rabbits in their metabolism cages at least 3 d before the injection and replace drinking water by the iodide/saline solution described in (4) above. This substitution is maintained throughout the experimental period.
(2) Prepare the rabbit for injection and bleeding by shaving the hair above the marginal vein of both ears.
(3) Inject 4-5 ml of the labelled albumin solution into the marginal vein of one ear of the rabbit and note the time of injection and prepare a standard by diluting about 1 ml[12] of the labelled albumin to 20 ml with diluted NaOH.

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[12] It is much more accurate to measure the amounts of labelled albumin injected and used for the standard by weight rather than volume. This is easily done by weighing the syringe full and again after delivering either into the rabbit or the volumetric flask used for the standard.

(4) Five minutes after the injection collect a heparinized blood sample (3-5 ml) from the marginal vein of the ear not used for injection. Collect a further blood sample at the same time each day for a period of 10 d.
(5) Separate the plasma from each blood sample by centrifugation.
(6) Make complete 24-h collections of urine and fæces starting at the time of the first blood sample and continue daily throughout the experiment.

Preparation and counting of samples

(1) Pipette into counting tubes duplicate 0.2 ml-samples of the plasma obtained 5 min after injection and make up to the standard volume for counting (say, 5 ml) with approximately 0.01N NaOH. For all later plasma samples, 1.0-ml lots are made up to 5 ml as above.
(2) Measure out duplicate 0.2-ml samples of the standard and make up for counting in the same way.
(3) Weigh the total collection of fæces for each 24-h period, spread the pellets on a large sheet of paper, mix thoroughly and collect at random in counting tubes triplicate 5-g samples. With a glass rod pack these down to the appropriate volume for counting, e.g. 5 ml.
(4) Measure the volume of urine in each collection, make up to a round figure with water and pipette out in duplicate suitable aliquots for counting.
(5) Using the well-type counter, determine the net counts per minute of all the plasma, urine and fæces samples. If they are not all counted at the same time, remember to repeat the counting of the standards on each occasion where counts are being made so that appropriate corrections can be applied for decay and variations in sensitivity of the counter.

Calculations

(1) From the cpm/ml of the standard solution, the weight of labelled albumin solution used in preparing the standard and the weight of labelled albumin solution injected into the rabbit, calculate the total activity injected in counts per minute.
(2) Calculate the plasma volume of the rabbit as follows:

$$V_p = \frac{\text{total activity injected (cpm)}}{\text{cpm/ml plasma}}$$

(3) Taking the activity per millilitre of the 5-min plasma sample as unity, express the activity of the later plasma samples as fractions of it and then plot the plasma activity against time as abscissa on semi-log paper. Compare the curves obtained from the normal and infected animals.
(4) For each 24-h collection period calculate the total activity F excreted in the fæces, and the total activity U excreted in the urine. From the plasma volume and the cpm/ml plasma at the beginning of each collection period, calculate the total plasma activity P. The fractional catabolic rate K is then given by $K = (U + F)/P$.

(5) Calculate K for each 24-h period throughout the experiment and compare the mean value for the normal rabbit with that for the infected one.

Questions

(1) Why is it apparently satisfactory in this experiment to calculate the plasma volume from the activity of a single post-injection sample (cf. Exercise 2)?
(2) What factors are likely to cause the day-to-day variations of K determined as above?
(3) What will be the effect on the results of a significant change in plasma volume during the course of the experiment?

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16. MEASUREMENTS OF THE SPECIFIC ACTIVITY OF EXPIRED CO_2 AFTER ADMINISTRATION OF ^{14}C-GLUCOSE TO RATS

Objective

To compare the utilization of ^{14}C-glucose in fasted and non-fasted rats by measuring the incorporation of ^{14}C into CO_2 and by determining the distribution of ^{14}C in the tissues.

Procedure

(1) Fast a rat for 24 h.
(2) Inject a rat intraperitoneally with 1 ml of a uniformly labelled ^{14}C-glucose solution.
    (a) This solution should contain about 6 μCi ^{14}C and 1 g glucose per millilitre;
    (b) At the time of dosing apply a sample of the dosing solution to filter paper and run the chromatogram.
(3) Place the rat in the metabolism chamber and measure the CO_2 and $^{14}CO_2$ output. Add 35 ml of hyamine solution to the gas-washing bottle before starting the measurement.
(4) After the peak of activity has passed, remove the animal from the chamber and sacrifice with nembutal.
(5) Pipette an aliquot (2-5 ml) of the hyamine solution from the gas-washing bottle into a counting vial and add 15 ml of scintillation fluid.
(6) Count in the liquid scintillation counter making all corrections needed.
(7) Repeat this procedure with the non-fasted rat.
(8) Compare: (a) time of peak; (b) overall specific activity; (c) total ^{14}C output.

17. AN OXIDATION TECHNIQUE FOR PREPARATION OF LIQUID-SCINTILLATION SAMPLES

Objective

To demonstrate the preparation by the bomb-combustion technique of samples for determination of ^{14}C content.

Procedure

Dose each rat with 5.0 μCi of uniformly ^{14}C-labelled glucose about 2 h before the experiment is to begin. At the end of this period sacrifice the animals by an overdose of nembutal and take the following tissues for analysis: blood (be sure to heparinize the syringe), liver (blot carefully to remove excess blood), and a piece of muscle. Place the tissue in a bag made up of dialysis tubing and dry under a heat lamp. The bag and contents should weigh not more than 300 mg wet weight.

The procedure for combustion is as follows:

(1) Place the bag in the sample basket of the flask, making sure that the black mark on the bag is plainly evident.
(2) Grease the stopcock of the flask with apiezon grease.
(3) Gas the flask with oxygen for 2 min.
(4) Place the sample container firmly on the flask.
(5) Place the flask in a dry-ice acetone bath for 30 sec.
(6) Remove the flask from the bath and place in the combustion chamber.
(7) Carry out combustion by means of infra-red lamp.
(8) As soon as the combustion is complete remove the flask from the chamber and place it in the dry-ice bath; leave it for 1 min.
(9) Remove the flask from the ice bath and add 15 ml of the ethanolamine[13] absorbing-counting solution to the side arm of the flask.
(10) Allow the counting solution to drain into the flask until air is heard running out; turn off the stopcock and allow the flask to set for 30 min at room temperature (swirl the flask frequently).
(11) Remove two 5-ml aliquots of this material and place in scintillation vials for determination of activity.

Calculations

Calculate per cent of dose per gram or millilitre of tissue.

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[13] Ethanolamine is an efficient and inexpensive trapping material and does not interfere with the liquid-scintillation process. A solvent mixture containing ethanolamine, ethylene glycolmonomethyl ether and toluene in a ratio 1 : 8 : 10 by volume has been found to be most suitable. The use of ethanolamine-ethylene glycolmonomethyl ether was required to facilitate the solubility of ethanolamine in toluene. For this system 5 to 6 g of PPO/litre is required.

18. DETERMINATION OF TOTAL-BODY WATER

The determination of total-body water volume in vivo is of interest in many studies. Many tracers have been used, but the most common is tritiated water or THO (^{3}HOH). The experimental method is simple and a classical example of tracer dilution.

Materials and equipment

(1) Tritiated water – high specific activity;
(2) Experimental animals (dog, goat, sheep, rabbit, etc.);
(3) Liquid scintillation counter;
(4) Physiological saline.

Procedure

(1) The tritiated-water dose to the animal should be approximately 5 mCi per 100 kg body weight. The specific activity should be approximately 5 mCi/ml.
(2) Pipette the dose into a polyethylene beaker. Pipette an aliquot of the dose into a liquid scintillation vial.
(3) Take up the dose into a syringe (10-25 ml volume).
(4) Wash the beaker with 1-2 ml of physiological saline and take up in the syringe.
(5) Repeat the washing process 2-3 additional times until it is assured the entire dose is in the syringe.
(6) An indwelling jugular cannula may be inserted into the jugular vein of the animal. A "Tee" syringe fitting may be adapted and a syringe with physiological saline attached to one connection.
(7) Attach the syringe containing the THO to the other "Tee" connection and inject into the animal. Wash the dose syringe with saline from the other syringe and inject into the animal. Repeat until it is assured that the entire dose has been delivered intravenously.
(8) Take blood samples at 5 min, 10 min, 30 min, 60 min, and 2 h, 3 h, 4 h and 6 h after administering the dose. (If possible continue sampling for 2-3 days. The animal must be on a constant diet and water intake.)
(9) Treat the blood samples as in experiment 12 for conversion to water and count in a liquid scintillation counter. Count the dose aliquot.
(10) Plot the specific activity versus time on semi-log graph paper. Extrapolate the straight-line portion of the curve through the last 3 or 4 points to zero time.
(11) Calculate total-body water in the animal by

$$\text{Total-body water} = \frac{r_{\text{dose}}\ (\text{cpm})}{a_0(\text{cpm/ml}}$$

where r_{dose} = net count rate of injected dose
a = extrapolated value of specific activity at zero time.

(12) What is the biological half-life of water in the animal? Calculate the turnover rate.
(13) Calculate total-body fat by the assumption that total-body water is 73.2% of the fat-free body weight.
(14) Consider all sources of error in the experiment.

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19. DETERMINATION OF TOTAL-BODY SODIUM AND EXCHANGEABLE SODIUM IN THE RAT

Introduction

Total-body sodium (Na_T) may be determined in vivo by neutron activation of ^{23}Na to ^{24}Na and subsequent whole-body counting. Exchangeable sodium (Na_e) may be determined independently in vivo by tracer dilution using ^{24}Na (or ^{22}Na). Total-body sodium must by definition be greater than exchangeable sodium. Na_e represents the osmotically active Na in the body, principally in extracellular fluid, and usually Na_e/Na_T will be about 0.8. There is a "non-exchangeable" pool of sodium principally in the skeleton and this is a function of age and body composition. This exercise is included to demonstrate the techniques to measure these parameters in vivo.

A. Determination of total-body sodium

Materials and equipment

(1) Rats
(2) Reactor or neutron source capable of producing thermal flux at least 10^6 n/cm^2 sec
(3) NaI(Tl) scintillation crystal-spectrometer
(4) Rat holder and rat phantom.

Procedure

(1) Weigh the rat.
(2) Construct a rat holder of lucite or perspex similar to that shown in Fig. 42. Cylinders of different diameter may be used for rats of different weight.
(3) Rat is inserted head-first and secured by a wad of kleenex and tape.
(4) Prepare a rat phantom from a polyethylene bottle selected to be as identical as possible in size and shape to the rat holder.

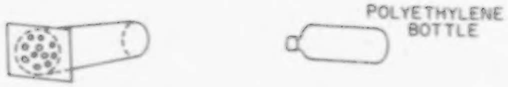

FIG.42. Rat holder and rat phantom.

(5) Add to the rat phantom an accurately weighed amount of NaCl, approximately 2 g. Fill with distilled water and seal.
(6) Irradiate the rat to the thermal neutron flux for approximately 1 h, but accurately record the time length of irradiation.
(7) Irradiate the rat phantom in the identical position for the same length of time.
(8) Count both the rat and the rat phantom by the NaI(Tl) crystal-spectrometer at approximately the same time after their respective irradiation. Determine the count rate by integration of the area under the 1.38-MeV total absorption peak on the gamma-ray spectrum.
(9) Calculate the weight of Na in the rat by the following proportion

$$\frac{\text{weight of Na in rat}}{\text{weight of Na in phantom}} = \frac{r_{rat}}{r_{phantom}}$$

where r = net count rate.

(10) Express Na_T as % of body weight. The value should be approximately 0.14%.
(11) From inspection of the gamma-ray spectra, what other radionuclides were produced in the rat by the neutron irradiation? Could total body Cl, Ca or K be estimated by the same technique?

B. Determination of exchangeable sodium in the rat

Materials and equipment

(1) Rat — preferably the same rat as used in part A of this experiment.
(2) ^{24}Na solution of high specific activity.
(3) Rat metabolism cage.
(4) NaI(Tl) scintillation crystal-spectrometer.
(5) Flame photometer.
(6) Counting vials (20 ml) of the liquid-scintillation-counter type.

Procedure

(1) Inject a known volume of ^{24}Na solution (approximately 2 μCi) intraperitoneally into the rat. Put the rat in the metabolic cage and begin urine collection.
(2) Inject an identical volume of the dose into a liquid-scintillation counting vial. Make up to 15 ml with water.
(3) After at least 6 h withdraw approximately 1 ml of blood from the rat by heart puncture or from the tail vein.

(4) Centrifuge the blood and pipette an aliquot of plasma into a counting vial. Make up to 15 ml with distilled water and count on the scintillation crystal.

(5) Determine the Na concentration in the plasma sample by flame photometry and calculate the specific activity.

(6) Count the vial containing an identical volume of the dose.

(7) Wash down the metabolic cage with water into the urine-collection vial. Make up to 15 ml and count.

(8) Correct all net count rates to an arbitrary initial time.

(9) Calculate Na_e as follows

$$Na_e = \frac{r_{dose} - r_{excreted}(\text{cpm})}{a\ (\text{cpm/mEq})}$$

where r_{dose} = net count rate of dose
$r_{excreted}$ = net count rate of dose excreted
a = specific activity of plasma in cpm/mEq.

(10) If part A was completed, calculate the ratio Na_e/Na_T.

(11) How can it be proven that the non-exchangeable pool of Na is in bone?

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